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Edited by

P. H. GREGORY and W. C. MOORE

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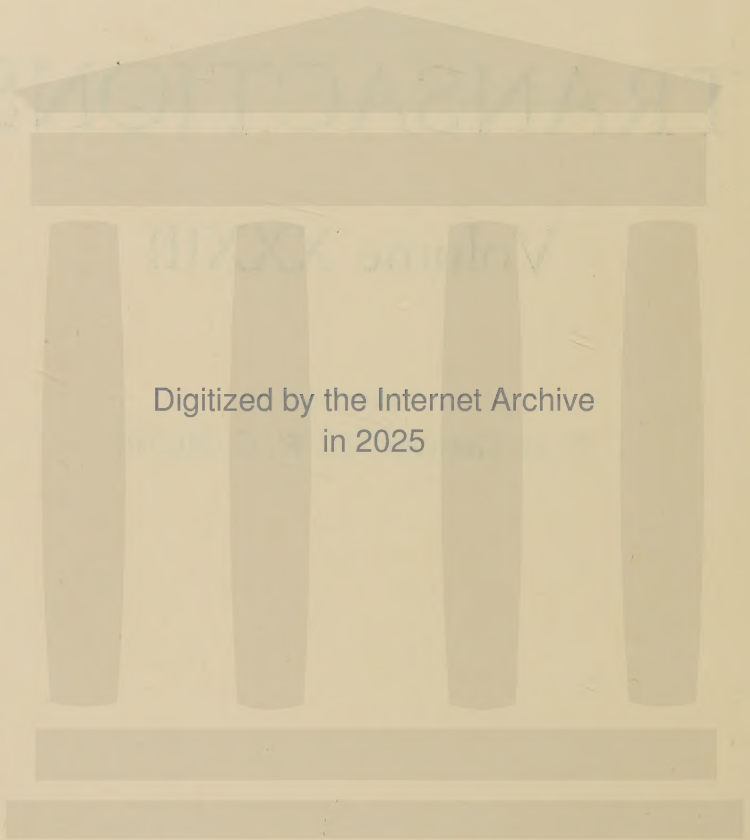

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President, 1948

PRESIDENTIAL ADDRESS

SEED-BORNE FUNGI AND THEIR SIGNIFICANCE

By A. E. MUSKETT, D.Sc., A.R.C.S., M.R.I.A.

In this essay the term seed is used in its broadest sense, suggestive of its functional rather than its true botanical meaning. Its use covers, therefore, not only the true seed but all those organs or parts of organs of plants which serve to propagate the species. The proposition is, then, to study the fungi which may be found occurring in, on, or in association with any part of a plant which may be used for propagation purposes, and to discuss their significance. It is by no means unnatural that fungi of many and diverse types should be present in a living state in such an environment. Fungi, together with the bacteria, with which they are so closely allied in function and from which they have been divorced too long in study circles, are the natural cleansing agents of the organic world, where they perform a work of vital importance in relation to which their modest proportions and insignificant appearances are quite out of keeping. The realm of mycology and bacteriology has only become really known—and even now how incompletely known—through the aid of modern microscopy and, in spite of the vast amount of ready knowledge which has accumulated in the present century, a yet vaster store remains to be explored which, when tapped, will provide substantially not only to the common pool of knowledge, but also to the service of world economy to which scientific discovery has already brought revolution in the present age. Normally, fungi and bacteria will be found in association with any dead or decrepit matter upon which they depend for their livelihood, and wherever such material exists, together with moisture and oxygen when necessary, there will be found specimens of mycological and bacteriological interest. And where is there a seed or planting unit which will not have somewhere a proportion of such dead and decrepit matter, whether it be testa, glume, pale, bark, scale leaves, or even some dead attachment foreign to itself? It is here that so many of these organisms are most commonly found.

Fungi and bacteria may be present on or in these tissues in an actively growing form or, under dry conditions, as thick-walled resting mycelium, chlamydospores, or resting spores—dormant, but waiting the access of moisture and warmth in order to grow and develop. Or fungi may occur on the seed as spores, arriving as wind-borne strangers and not engendered in the tissues but merely close adherents, their future staked with that of the seed they have reached willy-nilly. Or again, they may occur in association with the seed, not even touching it, but ensconced in a fragment of soil or vegetable detritus accompanying the seed and with which it will be sown. There remains yet another way in which a fungus may be seed-borne and this is as living mycelium within the internal tissues of the seed itself. In

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this case the relationship between the seed or planting unit and the fungus may be highly specialized. The fungus, more often than not, is parasitic and is frequently dependent for its existence upon the plant species which it inhabits. So a fungus may be borne with, on, or in the seed or planting unit, and the significance of its presence will frequently depend upon the way in which it is carried.

Generally speaking, the seed-borne fungi cannot be said to belong to any particular class or group, although instances can be cited where there is a close and often very specialized affinity between the members of an alliance and the plants whereon they live (e.g. the cereal smuts). The whole gamut of the fungus range with its great diversity of form is involved. Internally borne Phycomycetes—especially the Oomycetes—are well represented in planting units used for vegetative propagation. Here the organism is normally parasitic and occurs as living mycelium or as oospores within the tissues: e.g. *Phytophthora infestans* (Mont.) de Bary and the tubers of *Solanum tuberosum* L., *Phytophthora fragariae* Hickman and the rooted runners of *Fragaria* spp., *Peronospora destructor* (Berk.) Casp. and the sets of *Allium* spp. For the Zygomycetes no better example can be chosen than those species of *Mucor* which occur with such frequency on the dead or moribund tissues of the true seed. *Phialea temulenta* Prill. & Delacr., species of *Sclerotinia* and its imperfect form *Botrytis* represent the Discomycetes and provide some excellent examples of seed-borne parasitic fungi. Species of *Penicillium* and *Aspergillus* occur regularly as common moulds on seeds. The Pyrenomycetes are well represented by such parasitic forms as *Claviceps* spp. and *Epichloe typhina* (Fr.) Fr. with its mycelium perennial in the rhizomes of *Dactylis glomerata* L. etc. The *Helminthosporium* stages of species of the genus *Pyrenophora* are prominent as seed-borne parasites of the Gramineae while, if all the varying seed-borne forms of *Fusarium* are regarded as phases of the genus *Nectria* and its allies, then the Pyrenomycetes are indeed important, for the further study of seed-borne Fusaria may possibly prove to be one of the most interesting lines of investigation in this field. *Chaetomium* spp. occur commonly on the glumes and pales of cereal seeds, particularly oats, and appear to thrive on this cellulosic substratum. *Nectria rubi* Osterw., causing as it does the crown rot of raspberry, may be an example of a Pyrenomycete carried and spread in planting stocks of raspberries. The Ustilaginales provide the most classic example: the seed-borne nature of a number of species of this alliance is knowledge of long standing and has received a wealth of attention. In fact it is not an exaggeration to say that for some time the mention of seed-borne parasitic fungi suggested immediate reference to the cereal smuts and to very little else. The Uredinales cannot be excluded, and the rust of flax, *Melampsora lini* (Ehrenb.) Lév., must, unless and until the present view is contradicted, rank as an outstanding example of a parasite carried not on or in the seed but as teleutosori present on minute fragments of crop detritus which occur in the seed sample. But the rust fungi may also be carried by vegetative planting units and mint rust—*Puccinia menthae* Pers.—with its mycelium perennial in the rootstock may be cited in this connexion. Even the Autobasidiomycetes are represented, for *Corticium solani* (Prill. & Delacr.) Bourd. & Galz. is commonly found in its *Rhizoctonia* phase. *Armillaria mellea* (Fr.) Quél. cannot escape attention as

a possible contaminant of planting material even if not carried by the true seed. The Fungi Imperfecti offer some of the best-known examples and the mention of *Septoria apii* Chester, *Colletotrichum linicola* Pethybr. & Laff., *Phoma lingam* (Fr.) Desm., *Ascochyta pisi* Lib., *Polyspora lini* Laff. and *Alternaria* spp., to quote but a few, suggests their great importance as seed-borne pathogens. For the Actinomycetes, *Actinomyces scabies* (Thaxt.) Güssow carried by the potato tuber and causing common scab will suffice. The Plasmodiophoraceae contains *Spongospora subterranea* (Wallr.) Lagerh., which is also carried by the potato tuber, and *Plasmodiophora brassicae* Woron., claims for the carriage of which by the true seeds of the Brassicae have recently been made. And then there are those fungi as yet unnamed—the darnel fungus for instance which inhabits the seeds and system of darnel grass (*Lolium temulentum* L.). That bacteria are seed-borne in profusion is indeed common knowledge and *Pseudomonas coronafaciens* (Elliott) Stapp, the cause of halo blight of oats, furnishes an example, but far too little is known of this aspect of the problem and it is undoubtedly one to which much more attention will have to be given in the future.

The value of the study of seed-borne fungi as an aid to systematic mycology is something which should not be overlooked. An impetus to more intensive taxonomic studies is urgently required and the fungus flora of seeds offers scope to those workers who would select this field for their activity. True seeds can usually be relatively easily handled and preserved and offer many advantages to the observer. The necessity for observing the behaviour of fungi in their natural habitat for taxonomic purposes must be stressed, and it would seem that species occurring on or growing directly from the seed show considerable stability as compared with the variations in form so often encountered in the same organisms when grown continuously on culture media. The true seed may become an even more attractive material for culture work should the method of sterilization by a fumigant such as propylene oxide be perfected. Apart from species of *Penicillium* and *Aspergillus*, *Alternaria tenuis* and moulds commonly occurring on a wide range of seeds, many species of fungi are confined to the seeds of a small group of plants or even to those of one plant, so that the ecology of seed-borne fungi is a subject not without interest. The systematic study of these fungi has an additional advantage in that the information so obtained is likely to have an immediate applied value. A detailed knowledge of the fungus flora of seeds may be of considerable assistance in helping to determine the place of origin of a particular seed sample. For instance, it is already known that a sample of wild white clover seed contaminated with *Sclerotinia trifoliorum* Erikss. is most likely to have been produced in New Zealand; that fibre flax seed produced in Canada is frequently heavily contaminated with *Alternaria* spp., and that a sample of fibre flax seed produced in the United Kingdom and heavily contaminated with *Colletotrichum linicola* will have been produced in the north-western districts of the country. The presence and degree of prevalence of certain moulds on seed samples may provide useful information concerning the conditions under which the seed was processed and stored. Findings such as these are definitely helpful and can be of great service in furthering the practice of the production of high quality seed.

It may be asked why it is that so much attention should be given to seed-borne fungi—whether it is that things were always as they are and that the greater interest shown is mainly due to the increase in general knowledge of the problem, or whether the incidence of these organisms on seeds and planting stock, both as parasites and saprophytes, is increasing and so presents a new and somewhat novel situation. The answer seems to lie in both of these two possible explanations. That there has always been a common and abundant fungal and bacterial flora of seeds and planting stocks must be true, but it is only by the use of modern equipment and the application of scientific method that it has become possible to examine and understand this flora, and so account, in a reasoned manner, for many of the phenomena explained away traditionally in the past. At the same time, there can be no doubt that this flora is changing; is possibly becoming more varied, and that the changes which have taken and are still taking place have produced a situation which demands serious attention. The movement and transport of seeds and planting stocks throughout the world has never been more easy or pronounced than at the present time. This practice is not only based on trading economy but also on the fact that the introduction of fresh seeds and planting stocks from one region for use in another has frequently proved very beneficial to the crop. At the same time the plant breeder is continually introducing new and improved varieties of crop plants, and such changes have been made that it is often difficult to recognize the produce of one hundred years ago when compared with that of to-day. Systems of cultivation have also tended to become much more intensive and huge areas are now frequently devoted to the intensive cultivation of a single crop, with often far too little regard for crop rotation. All these changes and developments will be reflected in the fungus and bacterial flora of seeds and planting stocks as used in present-day agriculture and horticulture. Not only are seeds and planting stocks transported from region to region but so also are the micro-organisms present upon them and in them. The seed-borne fungus and bacterial flora is rapidly being spread throughout the world usually unnoticed and unfettered by the most alert customs post. Some species will undoubtedly succumb and perish in their new surroundings but others will flourish and may even thrive more successfully in the land of their adoption. New varieties of plants introduced on account of their possessing more desirable qualities may attract a new flora of micro-organisms unknown in the older types, while changes in the quantity and quality of the flora may also be expected under systems of intensive propagation and cultivation. Man has never interfered more with nature's nicely adjusted balance than he is doing to-day and changes observed in the micro-flora represent but one aspect of this disturbance. For a time all may seem to go well but ultimately there is often a price to be paid for such interference, and the price in agriculture and horticulture will largely be paid by the necessity for developing the science of plant pathology and its kindred sciences in an attempt to restore the balance, the original upset of which passed unobserved. Thus, the application of scientific method partly explains that which remained hidden in the past, and partly the new and ever-changing situation brought about by altered conditions and the power of the hand of man upon his world.

What is the effect of the presence of micro-organisms on the seed; how may they affect the life of the germinating seed and that of the plant it will produce: are they beneficial or harmful? Many seed-borne fungi are virulent parasites with their existence closely linked with that of the host plant; some behave as parasites under certain conditions; others appear to exert no particular influence one way or the other, while a few must be regarded as beneficial.

In the Orchidaceae it is well known that the seeds will not usually germinate to produce satisfactory plants without the presence of a mycorrhizal fungus which is not seed-transmitted, but in ling (*Calluna vulgaris* L.) when the presence of a fungus is also essential for healthy development, the fungus is transmitted with the seed. The nodule bacteria are essential for the healthy development of the Leguminosae and in lucerne (*Medicago sativa* L.) the precaution is frequently taken of inoculating the seed with a culture of the organism in order to ensure it being present. But the known cases where a seed-transmitted micro-organism is helpful or essential for growth and where it may even be added to ensure good growth, are few. In these instances the full function of the organism is not properly understood, and it may be that they represent instances of the most highly specialized form of parasitism where the interrelationship of host and parasite has become so delicately balanced as to produce a state of interdependence assuming true symbiosis.

Other instances exemplifying a very close relationship between plant and fungus are those of darnel grass (*Lolium temulentum* L.), and cocksfoot (*Dactylis glomerata* L.), in both of which the fungus is systemic and transmitted through the seed. In cocksfoot the fungus is the parasite *Epichloe typhina* (Fr.) Fr., responsible for the disease called choke, while in darnel grass it has been suggested that it may be a member of the Ustilaginales. The significance of the reported occurrence of internal mycelium in plants and seeds, especially in the Gramineae, is something about which more is likely to be heard in the future, and it is of interest to note that suggestions have been made as to a possible linkage between the presence of internal mycelium in grasses and their toxicity to livestock.

The cases where the presence of a seed-borne micro-organism is harmful and may seriously endanger the health and life of the plant are innumerable. *Tilletia caries* (DC.) Tul., the cause of bunt or covered smut in wheat, is a typical example of a parasitic fungus carried as spores on the outside of the seed. The actual health of the contaminated seed is not endangered and infection occurs in the young developing plant. As the seeds of an infected plant are totally destroyed they do not carry the fungus in the true sense but their remains—as 'bunt balls'—serve as foci for the distribution of the chlamydospores of the fungus to the healthy seeds produced by unaffected plants. In *Ustilago avenae* (Pers.) Rostr., the cause of loose smut of oats, and in species of *Helminthosporium* (*Pyrenophora*) responsible for stripe diseases in the Gramineae the fungus may not only be carried as spores external to the seed but also as mycelium produced in the glume tissues and in the outer layers of the caryopsis. Here again the health of the seed as such is not endangered and it is only when growth has commenced that the developing seedling becomes subject to infection. In the stripe diseases the seeds pro-

duced by plants which have not been killed or crippled by the disease, develop normally and become contaminated by spores produced by secondary lesions on the leaves. *Colletotrichum linicola*, the cause of seedling blight, and *Polyspora lini*, the cause of stem break and browning in flax may offer almost parallel examples to the stripe diseases of cereals, but here the actual health of the seed may be endangered. The fungus may attack the living seed itself and much depends upon the stage reached in seed development when contact is made with the fungus. If, through the medium of the seed boll and the funicle, the fungus reaches the young immature seed then the embryo may be attacked and killed. But if the seed is mature at the time it will probably escape injury, the fungus will be carried in the mucilaginous external layers of the seed coat and the danger of infection will lie with the young developing plant. Many of the seed-borne parasitic fungi fall into this category, where they may not only be carried on or in the external tissues of viable seeds but where they may also attack and destroy the seeds themselves. The details will vary from case to case and each instance must be investigated and considered on its own merits. *Phialea temulenta* causing blind seed disease in ryegrass may perhaps be quoted as an extreme case where, although the fungus may be carried by viable seeds, it is more usual for it to attack and destroy the seed. A wide range of types of seed contamination and infection frequently depending upon the seasonal conditions prevailing, may be found to occur in any one particular example of the group. This factor is of some importance, for when studying disease control it may have a bearing upon the efficacy of seed disinfection.

No better example of a parasitic fungus borne internally within the tissues of the seed and causing no damage to the seed can be cited than that of *Ustilago tritici* (Pers.) Rostr., the cause of loose smut of wheat. Here the mycelium occurs within the grain and causes no immediate injury to the seed, which germinates in a normal manner. The infection is carried into the plant and no damage occurs until the time of flowering, when the fungus bursts into activity, destroys the spike and produces its mass of chlamydospores.

Flax rust—*Melampsora lini*—furnishes one of the best examples of the carriage of a fungus with the seed, for in this case the seed itself remains not only unharmed but uncontaminated. The fungus occurs in crop detritus present in the seed sample, although minute fragments of such infected detritus may adhere to the coat of the flax seed.

There remains a further group of seed-borne fungi which may be generally described as moulds and brief reference to which has already been made. They are species of such genera as *Aspergillus*, *Penicillium*, *Mucor*, etc., which although not classed as virulent parasites, may on occasion harm the seed. The chief damage they cause is probably in seed which has been injured during processing and which has thereby become cracked or broken. With the advent of moist warm conditions these fungi develop, effect an entry through injuries or wounds and live on the food stored as endosperm for the young plant, or become feebly parasitic on the seedling, especially if a portion of the embryo has been injured in the processing. Adverse weather conditions at the time of germination may react unfavourably upon the seedling growth and thereby encourage attack by such fungi. Or it is possible that by the production of toxic substances in the course of

their normal metabolism, these fungi may poison the developing plant and thereby kill it or detract from its vigour. At this point it may not be out of place to mention the possible danger of using seeds contaminated with fungi as food for livestock. The case of seed barley affected with *Gibberella zeae* (Schw.) Petch may be quoted, where the fermentation processes set up in the preparation of barley meal produce toxic substances poisonous to pigs.

In any consideration of the action of seed-borne fungi and bacteria the possibility of growth antagonism existing between various species of these organisms must not be overlooked. That such antagonism exists between micro-organisms has been known for a long while, although it has only recently become news on account of the sensational behaviour of substances like penicillin and streptomycin. Further studies of the behaviour of micro-organisms carried by the seed may reveal instances where the growth of a harmful species is suppressed by that of one which is harmless. Should such a finding be made and utilized it could have far-reaching effects, but until this has been done the possibility must remain in the field of pure speculation.

The ways in which seed becomes contaminated with micro-organisms are manifold and in many instances are but imperfectly understood. In the cereal smuts the picture would seem to be fairly clear and does not call for comment. In *Helminthosporium* disease of oats and some other cereal leaf stripes the seed derives its contamination from spores produced on the secondary lesions of the leaves of a maturing crop. *Colletotrichum linicola* and *Polyspora lini* present interesting examples in flax. Here, contaminated seed gives rise to diseased seedlings and as the crop grows the fungus gradually spreads to the higher leaves so that the disease tends to keep pace with the crop growth. After flowering and as the bolls mature, the fungus attacks the dying sepals, passes on to the bolls which it penetrates and so reaches the seed by way of the funicle. These stages of infection are not always easy to follow and, frequently, heavily contaminated seed samples are derived from crops in which it is difficult to find more than a trace of the disease. In this case crop inspection is, therefore, by no means an infallible guide to the extent to which the seed produced may be contaminated; this can only be properly ascertained by laboratory examination of the seed. The species of *Phoma* responsible for foot rot in flax is probably contracted from the soil in the first instance, and in such cases the disease is seldom serious unless flax has been grown so frequently in the same land as to allow of a heavy inoculum being built up in the soil. But crops so affected usually produce a proportion of contaminated seeds and it is at this stage that the disease may assume a serious aspect. Seed-borne *Phoma* appears to be a much more certain way of producing foot rot and if heavily contaminated seed is sown there is a real danger of the disease becoming epidemic.

Apart from the methods by which seed may become contaminated during the course of the normal growth of a crop, there are two other factors which must be taken into account in considering the methods of seed contamination. These are the effect of crop weathering and the spreading of micro-organisms during the operations of harvesting, stacking, threshing, processing and storage. Ample opportunity for contamination will occur in lodged and weathered crops. The fact that under such conditions wet

weather frequently prevails and the seed-bearing portions of the crop are brought into close contact with the undergrowth and even the soil itself, emphasizes the danger to which the seed becomes exposed. Again, during the processes of harvesting, stacking, threshing and processing there will be ample opportunity for the dispersal of spores of fungi, produced on the vegetative portions of the crop, being spread to the seed. If conditions in the stack happen to be moist then the period of stacking may serve as one of incubation and the spores formed under such conditions will be spread during de-seeding and processing. In this way a great deal of external contamination of the seed with fungus spores is likely to occur. Similarly, the dispersal of mould spores throughout the seed will depend to some extent upon the cleanliness of the premises in which processing operations are carried out. Unclean, ill-kept premises may serve as an incubation centre for moulds with the result that their use may be responsible for heavy mould contamination of the seed. The handling of crops in sequence may also result in the organisms contaminating one crop being spread to others quite free from contamination before handling. Storage conditions will determine largely whether or not the danger of contamination is likely to be increased during the period between processing and sowing. Under damp warm conditions there is little doubt that seed with a moderate moisture content will tend to become more highly contaminated. In such cases the spreading of contamination even during the operation of sowing must be taken into account.

The fact that seed is contaminated with a fungus parasite does not necessarily mean that the resultant crop will be diseased. For the parasite to be able to attack, a third factor calls for consideration and this concerns the growth conditions prevailing at the time. In order to set light to a piece of paper by the aid of a live match a striking medium must be available in order that the match may be struck. Thus, if the fungus parasite is likened to the match and the plant to the piece of paper, then the prevailing conditions of growth may be likened to the striking medium and will determine whether or not the fungus will attack and cause disease. For instance, a sample of seed oats heavily contaminated with *Helminthosporium avenae* Eidam may be sown under conditions which will result in the production of an almost healthy crop or where the crop of seedlings will be almost totally destroyed. Generally speaking, the conditions favouring the rapid and vigorous growth of the crop will be those which will secure freedom from attack by the disease. Under conditions of relatively high soil temperature and an adequate soil moisture content the oat plant will win and the fungus lose, so to speak. But if the soil temperature is low and the moisture content high or if the soil temperature is high and the moisture content low, then the crop will be attacked and succumb to the disease. In practice the most likely chance of securing conditions favourable to the growth of the oat plant and so avoiding attack by the fungus, will be obtained by late sowing, but as late sown crops are much more liable to attack by frit fly (*Oscinella frit* L.) the practice cannot be recommended without reserve. So, with the seed-borne fungus parasites of flax and with the cereal smuts, given the presence of parasite and host, the incidence of disease will be conditioned by the environment provided for the germination of the seed

and the growth of the plant. Similarly, the extent of the occurrence of seed diseases and seed contamination will largely depend upon weather conditions. Given a crop of ryegrass and an abundant inoculum of the ascospores of *Phialea temulenta*, wet and cool conditions at the time of flowering of the crop will be necessary to ensure an outbreak of the disease in epidemic form. Varietal characteristics of the crop plant may also play an important part in determining the incidence of disease. Those varieties or strains of ryegrass in which the flowers remain open for a longer period than usual and where the flowering period of the crop is extended, are more likely to contract the disease than others. Nor must the mutability of the fungus parasite be over-looked. Perhaps it is the very simplicity of the organization of the fungal thallus which makes its study so difficult. In the extreme a fungal hypha may be likened to a slender tube filled with vital matter containing a highly organized enzyme complex. A slight alteration in this complex and the nature of the organism may undergo a radical change. The effect of nutrition, irradiation, heat; any of these may effect such an alteration and the possibility of a saprophyte developing parasitic tendencies under such conditions must not be overlooked. That some slight change in the characteristics of the host plant due to varietal differences, age, or mode of nutrition may provide a fungus with greater or lesser opportunity is equally true. The conditioning of the incidence of disease by growth factors is a matter of the greatest importance for if this were not so, it would scarcely be possible to grow many crops successfully owing to the danger of disease. At the same time unnecessary risks must not be taken because the use of contaminated seed and planting stocks presents a real danger should the requisite combination of growth conditions occur, and the conscious adoption of such a practice cannot be regarded as anything other than a deliberate gamble.

Although there are cases where the presence of micro-organisms with seed may be beneficial or even essential, the reverse is generally the case and the contamination of seed and planting stocks with fungi and bacteria must be regarded as an undesirable feature. So it follows, in the present state of knowledge, that the best seed and planting stock will be that which is most free from such contamination and the steps which must be taken in order to ensure the good health of seeds and planting stocks will now be considered. But before doing this it will be well to consider briefly another group of pathogens affecting vegetative planting stocks more commonly than true seeds but without mention of which any such consideration would be incomplete. Whether or not the viruses are to be regarded as living organisms does not fall within the scope of this discussion, but as the technique employed in the investigation of virus problems is so closely akin to that used in bacteriology and mycology and as viruses may be a serious contaminant of planting stocks, any consideration of the health of such stocks must also include virus infection.

In the first place it is highly desirable that as much detailed knowledge as possible of the fungi, bacteria and viruses contaminating seeds and planting stocks be made available. Reference has already been made to the importance of this study. Such knowledge is fundamental and much of what can be done in practice to ensure the production of healthy seeds

and planting stocks will be dependent upon accumulated knowledge of this kind. Attempts have been made in some countries to prepare lists of recorded seed-borne parasitic fungi, but this is not sufficient. An important step forward will have been made when each country undertakes to publish a comprehensive list of the known contaminants of the seeds and planting stocks produced in that country, together with additional information concerning their behaviour under the existing climatic conditions.

The next step involves the provision of facilities for the adequate examination of samples of seeds and planting stocks for their contamination with micro-organisms and viruses. This work may necessitate the examination of some thousands of samples of seeds within a limited time and in order that this may be done, the availability of rapid and accurate technique for the purpose is essential. The technique employed in modern seed testing for determining the degree of purity and percentage germination of seed samples will serve as a guide here because if this work is to be undertaken on a large scale it will have to be carried out by trained assistants within time limits approximating those required in seed testing. An approach to this problem has been made in Northern Ireland, and laboratory technique has been devised whereby it is possible to examine seed samples of certain crops for the presence of seed-borne parasitic fungi within the time limits prescribed. This can now be done for *Helminthosporium avenae* in oats, *Colletotrichum linicola*, *Polyspora lini*, *Botrytis cinerea*, *Phoma* sp., *Fusarium lini* and *Alternaria* spp. in flax, and *Phialea temulenta* in ryegrass. Different methods have to be used for the seeds of different crops and it is as yet by no means clear whether different methods may not have to be used in some cases for different parasites occurring on seeds from the same crop plant. What is clear is that each organism must receive individual attention and the method used for the determination of its occurrence must be proved to be satisfactory before undertaking the work on a large scale.

With such technique available it is possible not only to examine specific seed samples when required but also to carry out national or international surveys of the seed produced from any particular crop with the object of determining its state of health. Such surveys carried out over a period of years should provide useful information by way of assisting with the production of healthy seed. The question as to whether the making of such surveys is likely to be worthwhile may, perhaps, best be answered by mentioning the results obtained by making a health survey of all fibre flax seed produced in the United Kingdom from 1943 onwards. These results have not yet been published but they may be summarized as follows. During each year since 1944 and commencing with the seed available from the 1943 crop, some 2000–4000 samples of flax seed produced in the United Kingdom have been systematically examined by the Ulster method for their contamination with seed-borne fungus parasites. The samples were collected in such a way as to be truly representative of the distribution of the crop over the whole country. The results obtained over a period of at least three years show clearly that seed liable to heavy contamination with *Colletotrichum linicola*, *Polyspora lini* and *Phoma* sp. is much more likely to be produced in the north-westerly districts of the country than towards the east and south. It may be said, in fact, that fibre flax seed produced in the

warmer and drier districts of east and south-east England is normally almost free from contamination. With *Botrytis cinerea* the results indicate that seed contaminated with this fungus is of more general occurrence and that climatic conditions do not exert the same controlling influence. When the severe crop damage which may be caused by seed-borne parasites is taken into consideration, the significance of possessing true knowledge of the health of a seed sample becomes apparent, and especially so if conditions are available for the production of seed free from contamination. From observations made up to date there would seem to be no doubt that east and south-east England are the most suitable districts for the production of healthy flax seed in the United Kingdom and with the aid offered by modern and intelligent seed disinfection as an additional safeguard, flax seed produced in these areas should be of as high a quality and as healthy as that produced anywhere, providing it has come from a rust-free crop. There can be no doubt as to the considerable value of this survey and there is no reason to suppose that equally interesting and useful results would not emerge from similar surveys made for the seeds of other crops. It is, perhaps, a pity that the flax-seed survey could not have been extended to include at least those other countries in western Europe which produce fibre flax seed, but some day this may be possible, for it is only because it is impracticable and not because it is impossible, that the political world cannot be considered as a whole in matters of this sort.

If work along these lines is carried further, its logical endpoint will be the provision of facilities whereby seeds and planting stocks can be systematically examined for contamination with fungi, bacteria and viruses with the object of determining their state of health. There is nothing particularly new in this idea, for provisions have already been made for the testing of seeds for their state of purity and percentage germination and the testing of planting stocks for the presence of viruses is steadily being developed. It simply means an extension of the provisions already made—with the emphasis on health.

But the provision of such facilities cannot be regarded as anything more than a means to an end. The ultimate aim must be to secure the highest possible yield of produce by providing ample supplies of healthy seeds and planting stocks. The costs of crop production have risen considerably; cultivation costs have never been higher and the economic significance of losses incurred through the use of poor quality seeds and planting stocks has never been greater. Moreover, the necessity for increased crop production to provide ample food supplies throughout the world is but an added reason for producing planting material of the highest quality. By helping in this way plant pathology can offer no greater service to humanity at the present time. In order to achieve the desired objective it will be necessary to devise and follow a carefully planned scheme leading towards the certification of seeds and planting stocks on a quality basis and towards this end the following suggestions are made:

(1) Wherever desirable nuclear stocks of seeds and planting materials known to be healthy should be raised and preserved with the object of providing a reservoir of high-grade produce to be used for the maintenance of the health of commercial stocks.

(2) Wherever possible seeds and planting stocks should be produced in those regions or districts best suited for the purpose and where the incidence of seed-borne parasites, whether fungi, bacteria or viruses, is likely to be negligible or low.

(3) Crops grown for the purpose of providing seed or propagating material should be regularly and properly inspected, one of the main objects of such inspection being to ensure the health of the crop and its freedom from seed-borne parasites in so far as this can be done by inspection in the field.

(4) Adequate facilities must be available for the laboratory and glass-house examination and testing of seeds and planting stocks as a final guarantee of their freedom from contamination with parasitic fungi, bacteria and viruses.

(5) Every advantage must be taken of the benefits attaching to modern methods of seed disinfection. Especially with seed-borne parasitic fungi and bacteria, the disinfection of seed with a selected therapeutant applied effectively at the correct dosage will prove an extremely valuable ancillary measure in ensuring a high standard of health.

(6) A comprehensive system of certification must be introduced in order that some warranty may be given of the quality of planting materials.

The introduction and maintenance of such a comprehensive scheme may appear at first sight to be uneconomic and something of an expensive luxury, but that this may not be so is shown by reference once again to the work undertaken in Northern Ireland in connexion with flax seed. It has been calculated that by introducing a certification scheme for flax seed during the period of the 1939–45 war whereby the health of the seed was systematically checked, heavily contaminated seed discarded for sowing purposes, and the whole of the seed stocks disinfected on a nation-wide basis, the crop of long fibre produced was increased by the value of at least £2,000,000. The entire cost of the work would have been covered by the saving of a few acres of crop each year.

In conclusion, one further thought. The complicated boundaries which have of necessity had to be evolved to preserve the political integrity of the strains and races of mankind do not appear to serve any special purpose in the case of micro-organisms. Apart from the limitations imposed by unsuitable environment, only the widest of the oceans would seem to provide anything like a satisfactory barrier against their penetration and infiltration so peaceful and so unobserved. It is clear, therefore, that problems relating to improvement in the health of seeds and planting stocks are international in character and will eventually have to be dealt with accordingly. This may be yet another means whereby thinking men and women may be brought together for the purpose of devising some improvement of the common lot, and thereby constitute but another example of the significant contribution which science can make to the humanities and so bring a measure of real relief to a muddled and bewildered world.

CALONECTRIA THEAE N.SP.—THE PERFECT STAGE OF *CERCOSPORELLA THEAE* PETCH

By C. A. LOOS, *Tea Research Institute of Ceylon*

(With 2 Text-figures)

The fungus which has been known as *Cercosporella theae* Petch (1917) causes a well-known leaf disease of tea in Ceylon (Petch, 1923) and South India. Gadd (1927) first reported the occurrence of a perithecial stage of this fungus in cultures, but gave no description beyond the observation that the perithecia are small, red or reddish yellow spheres about the size of a pin's head. Later, he reported the discovery of similar, but empty, perithecia on dead tea leaves (Gadd, 1929), and the presence of *C. theae* and *Calonectria* perithecia on diseased plants of *Tephrosia radicans* (Gadd, 1937). In South India, Subba Rao (1936) failed to obtain perfect perithecia in his cultures of *Cercosporella theae*, but later (1943) found in abundance, on the under surface of fallen tea leaves, sterile perithecial bodies which, when detached and placed in culture media, developed the characteristic spores of *C. theae*.

Recently, a species of *Calonectria* was found associated with a collar rot of *Crotalaria anagyroides*. On one plant, in addition to the red perithecia, were a few sporophores of what appeared to be *Cercosporella theae*. Attempts to obtain pure cultures from the conidia failed, but cultures from the ascospores all produced *Cercosporella* sporophores first and *Calonectria* perithecia later. This fact, together with the results of inoculation experiments, which demonstrated that both conidia and ascospores cause the same disease of tea, left no doubt that the conidia and ascospores belong to the same fungus. The question remaining was whether the fungus is *Cercosporella theae*.

The only observation that seemed to throw doubt on such a determination was the presence of globose, almost spherical swellings, 7–9 μ in diameter, at the ends of the main axes of sporophores in all cultures of the fungus from *Crotalaria* (Fig. 1 A). Similar axis terminations of *Cercosporella theae* had not been noted, and an examination of permanent preparations and fresh infected tea leaves showed the sporophore axis terminating in a narrow cylindro-clavate enlargement (Fig. 1 B–C.). This character appears to be constant, as cultures of *C. theae* from tea could readily be distinguished by it from cultures of the fungus from *Crotalaria*. A short comparative study of the two fungi was therefore undertaken, and the results are presented here. Before the study was completed the Crotalaria fungus was also found on diseased *Tephrosia vogelii* plants growing on the site previously occupied by the diseased Crotalarias. In culture the fungus behaved exactly as the fungus from *Crotalaria*, and further mention of this strain will not be made.

Cultures

Cultures of *Cercosporella theae* were obtained from conidia on infected tea leaves for comparison with the cultures already obtained from *Calonectria* ascospores from diseased Crotalarias.

For plate cultures Waksman's agar without acid, Quaker Oat agar and malt extract (Kepler's malt) agar were used. On these media *Cercosporella theae* formed little aerial mycelium, whereas the Crotalaria fungus produced it abundantly, sometimes filling the space between the agar and dish cover. Conidia were produced by both fungi on all media but always most abundantly by *C. theae*. In later subcultures of the Crotalaria fungus conidia formation ceased. Perithecia were formed by both fungi on Quaker Oat agar, by neither on malt extract agar, and only by the Crotalaria fungus on Waksman's agar. On Quaker Oat agar the perithecia were formed in large numbers, usually in clusters, on the agar surface after 3 weeks. The spores were discharged as a yellowish mass. The perithecia of the Crotalaria fungus were usually bright red, whereas those of *C. theae* were more orange at first, later becoming brownish red. Colouring matter was produced by both fungi; it varied from pale yellow at first, then to brown and finally to deep red brown. Its rate of formation and distribution also varied considerably; most frequently it occurred as radial streaks but sometimes in concentric zones as well. The colour did not diffuse readily through the agar, though agar in tubes was more evenly coloured than that on plates.

The two fungi were also grown on sterilized plant tissues; tea stems and leaves, stems of *Crotalaria anagyroides*, and leaves of *Acacia decurrens*. In general, the two fungi behaved alike; conidia were produced in large numbers for the first ten days and perithecia began to appear after the first week and discharged their spores after four weeks. Except on Acacia leaves the perithecia of the Crotalaria fungus were bright red, whereas those of *Cercosporella theae* were orange. On leaves of *Acacia decurrens* the perithecia of the Crotalaria fungus were orange red and those of *Cercosporella theae* light pink.

Cross inoculations

The Crotalaria fungus proved to be as virulent as the tea fungus on excised tea leaves, both when conidia and ascospores were used as inoculum. *C. theae* failed to cause infection of *Crotalaria anagyroides* seedlings when inoculated near the collar, except in one instance (of six inoculations) when the stems had been previously wounded. The Crotalaria fungus readily caused infections in both wounded and unwounded stems. Neither fungus caused disease on tea seedlings when infections were attempted at the collar. This is perhaps not surprising as neither fungus is known to cause a collar rot under field conditions.

The experiments showed that *Cercosporella theae* from tea is not pathogenic to *Crotalaria*, whereas the fungus from *Crotalaria* is capable of causing disease in both species.

Conidia

The conidiophores of both the tea fungus '*Cercosporella theae*', and the Crotalaria fungus consist of an erect main axis from one side of which arise a number of branches or metulae at irregularly spaced intervals. At the apex of each metula is a whorl of banana-shaped phialides, 13–15·5 × 2·9–3 μ,

which bear long multiseptate spores (Fig. 1).* Owing to the arrangement of the phialides the spores appear to be in bundles above which the main axis projects. The sporophores can be distinguished by the axis terminus,

Fig. 1. Terminal axis of sporophores. A, fungus from *Crotalaria*; B, *Cercosporella theae* from tea.

* A glance at the morphology of the conidiophore, as shown in Fig. 1, is sufficient to indicate that these conidial stages should not be included in the genus *Cercosporella*. The sterile main axis with terminal swollen head and the lateral penicillate conidia-bearing branches suggest the genus *Cylindrocladium*, but in that genus the spores are only 1-septate. The genus *Candelospora* Rea & Hawley, described in 1912 with the one species *C. ilicicola* Hawley, has 3-septate spores figured as borne in a manner closely resembling Petch's figure of '*Cercosporella theae*'. Unfortunately, no material of *Candelospora ilicicola* has been seen, but Fawcett & Klotz (in *Mycologia*, **29**, 213, 1937) have described another species of *Candelospora*, *C. citri*, on Citrus, which has conidiophores with the sterile, club-like main axis as in the fungi under consideration. We are informed by the Commonwealth Mycological Institute that Petch's fungus on tea has been there for some time regarded as a species of *Candelospora* and filed under that genus. Fortunately, since the perfect stage of the fungus is now known, there is no necessity to make a new combination under *Candelospora* for the imperfect stage.

that of *C. theae* being cylindro-clavate, whereas that of the Crotalaria fungus is globose (Fig. 1).

The conidia of *C. theae* vary considerably in length. Petch's (1917) measurements are 80–120 × 8 μ. Recent measurements of fifty spores from diseased tea leaves gave a range of 62–100 × 3·5–6·5 μ with a mean of 76·6 × 5·2 μ.

Conidia of the Crotalaria fungus from diseased Crotalarias measured 65–98 × 6 μ with a mean (of fifty) of 79 × 6μ, and so cannot be distinguished from *C. theae* by size. Numerous measurements of conidia of both fungi growing on different culture media were made and, as was to be expected, their means were found to vary with the substratum. In general, there was no significant difference between the mean measurements of the two fungi on any medium. On Waksman's agar the difference was most marked; the spores of the Crotalaria fungus measured 32–66 × 4–7 μ (mean 50 × 6 μ), whereas the conidia of the tea fungus measured 59–104 × 6 × 8 μ (mean 84 × 7 μ). On this medium the Crotalaria fungus formed relatively few conidia and many perithecia, whereas *C. theae* from tea formed many sporophores and no perithecia.

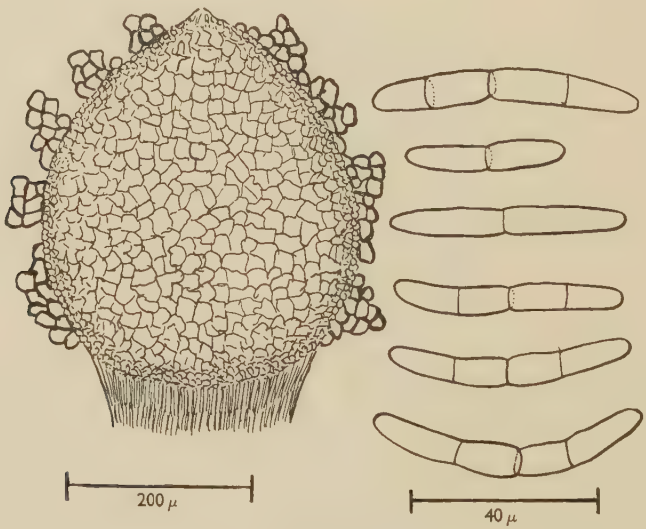

Fig. 2. *Calonectria theae*. Perithecium and spores.

Perithecia

The perithecia of both fungi are superficial, separate, globose and coloured. Usually the Crotalaria fungus has red perithecia, whereas those produced by the tea fungus are orange red. Both colour and size vary somewhat according to the substratum on which the fungi are grown, e.g. on sterilized leaves of *Acacia decurrens*, orange-red perithecia are formed by the Crotalaria fungus while those of *Cercosporella theae* are pink. On diseased plants of *Crotalaria anagyroides* the perithecia measured 320–420 × 330 μ (mean 390 × 330 μ); on sterilized Crotalaria stems the mean measurements were

484 × 393 μ, whereas the tea fungus perithecia were 364 × 337 μ. On sterilized tea leaves the perithecia of the tea fungus were the larger, whereas on Quaker Oat agar the perithecia of both fungi were approximately the same size.

The ascospores of both fungi are hyaline, elongate-fusoid, 1–3-septate, with a marked constriction in the outer wall at the middle septum (Fig. 2). The three septa are most apparent when the spore germinates. Ascospores from diseased Crotalaria plants measured 22–62 × 6–9 μ (mean of fifty, 48 × 7 μ) and when grown on other substrata the mean measurements varied from 42 × 5 μ on sterile tea leaf to 57 × 6 μ on sterile tea stems. The ascospores from the tea fungus were usually slightly larger than those of the Crotalaria fungus growing on the same medium. Mean measurements of the tea fungus varied from 53 × 7 μ on sterile tea leaf to 65 × 7 μ on sterile tea stems.

Conclusions

The fungus described by Petch as *Cercosporella theae* belongs to the form-genus *Candelospora*. The fungus from diseased *Crotalaria* differs from '*Cercosporella theae*' Petch chiefly in four main ways: (1) the termination of its sporophore axis is globose instead of cylindro-clavate; (2) its perithecia are a brighter red; (3) its ascospores are somewhat smaller; and (4) it is pathogenic to *Crotalaria anagyroides* as well as to tea. The two fungi, however, are so closely related that despite the differences enumerated above I regard them as two strains or varieties of the same species. For that reason I propose the name *Calonectria theae* for the perfect stage of Petch's fungus, and for the strain which attacks *Crotalaria* at the collar as well as the young leaves of tea the varietal name *crotalariae*.

Calonectria theae n.sp.

Perithecia superficial, globose to obovate, orange-red, 0·34–0·44 mm. in diameter, with base embedded in weft of orange coloured mycelium; wall parenchymatous with projecting cells giving a roughened appearance except for a small area around the ostiole which is smooth and naked; ostiole slightly elevated. Asci hyaline, 8-spored. Ascospores hyaline, elongate-fusoid, 1–3-septate, with a distinct constriction at the middle septum, 42–70 × 5–8 μ (mean of fifty spores 57·9 × 7 μ).

Imperfect stage '*Cercosporella theae*' Petch with the terminus of the sporophore main axis narrowly clavate.

On mature leaves of *Camellia theae* in culture.

Var. **crotalariae** n.var. Perithecia redder and spores smaller, 22–62 × 6–9 μ (mean of fifty spores 48 × 7 μ). Imperfect stage distinguishable by the globose terminations of the sporophore main axis.

On stems of *Crotalaria anagyroides* and *Tephrosia vogelii*.

Calonectria theae sp.nov.

Perithecia superficialia, basi in mycelio aurantiaco nidulantia, 0·34–0·44 mm. diametro, globosa vel obovata, aurantio-rubra, pariete pseudo-parenchymatico, externe ob cellulas prominentes rugosiusculo; ostiolum

leviter elevatum, disco nudo laevi circumdatum. Asci hyalini, octospori. Ascosporae hyalinae, elongato-fusoideae, 1–3-septatae, ad septum medium distincte constrictae, 42–70 × 5–8 μ (modus ex 50 sporis 57·9 × 7 μ).

Status conidicus sistit '*Cercosporella theae*' Petch, (= *Candelospora* sp.) conidiophorum axibus principalibus sursum anguste clavatis.

Hab: in foliis maturis *Camelliae theae* in vitro culta.

var. **crotalariae** var.nov. E typo differt peritheciis magis rubris, et sporis minoribus, 22–62 × 6–9 μ (modus ex 50 sporis 48 × 7 μ). Status conidicus quoque conidiophora axibus principalibus sursum globosis habet.

Hab: in caulibus *Crotalariae anagyroides* et *Tephrosiae vogelii*.

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Summary

Calonectria theae, the perfect stage of *Cercosporella theae* Petch, is described. A species of *Calonectria* which causes a collar rot of *Crotalaria anagyroides* and *Tephrosia vogelii* and has a *Cercosporella* imperfect stage is compared with *Calonectria theae*. Though the two fungi differ they are so closely related that they must be regarded as varieties of the same fungus. The Crotalaria fungus has been named var. *crotalariae*.

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FURTHER OBSERVATIONS ON THE SPORE GROWTH OF *EXOBASIDIUM VEXANS*

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In an earlier communication (Gadd & Loos, 1948) we gave evidence in support of the view that *Exobasidium vexans* produces basidiospores only, and we referred to the 'conidiophores' of Massee (1898) as sterile hyphae, whereas they are, of course, immature basidia. We also described the germination of the basidiospores, and stated that appressoria (haustoria) had not been observed. The formation of appressoria has not been reported by other observers, but we have now found that, given suitable conditions, they are normally produced in Ceylon, and that entry of the fungus into tea leaves is through the cuticle and not through stomata as previously supposed (Tunstall & Bose, 1921).

For our earlier studies we collected spores from the hymenial surface with a brush and germinated them in globules of water. In hanging drops the spores tend to come together in clumps which makes later observation difficult. Although clumping is largely eliminated by using 'standing' as opposed to 'hanging' drops, the results obtained are inconsistent and the germination capacity of the spores invariably appears to be low. Much better results are now obtained by using freshly discharged spores only, and by reducing water to a minimum.

The spores are not easily detached from the hymenium; they cannot be shaken or blown off, and spore discharge soon ceases when the diseased leaf is plucked. If, however, the severed leaf is supplied with water, discharge will continue for several days and spore prints are easily obtained. As the tea leaf has a very short petiole, it is convenient to remove a piece of stem with the diseased leaf and place its cut end in water. The leaf is then held horizontally above, but not touching, the glass slide or other surface on which the spore print is required. The spores adhere firmly to the glass or other surface and are not dislodged by running water, as from a tap, nor are they detached by washing in alcohol or xylol. The spores are therefore best germinated on the surface on which they are collected, to avoid damage by forcible removal. Overcrowding is in no way detrimental to germination, as the spores of a heavy deposit germinate as readily as those of a very light print.

The spores germinate best in the thinnest films of water such as are formed by condensation, and such films can be simulated with an atomizer. Because of the very small amount of water around the spore, particular care has to be taken against drying during germination.

With these improved methods, a high germination capacity of fresh spores and the formation of appressoria can be demonstrated. When the germ tubes have grown a short distance the tips enlarge to form spherical, somewhat dark coloured bodies 4·3–5·6 μ in diameter; and each spore may

form from one to four or five appressoria, depending on the number of germ tubes produced. In our earlier communication we described the flow of the protoplasm into the germ tube leaving the spore apparently empty. This flow continues into the appressorium so that when the appressorium is fully developed a great part of the germ tube is empty. Around the germ tube and appressorium is a film of mucilage which fixes them to the surface on which the spores germinate.

The speed at which appressoria are formed depends largely upon external conditions, including the nature of the surface on which the spores lie. On tea leaves, appressoria have been found within eight hours, and they are usually developed within twenty-four hours of the spores being wetted. The germ tubes then are very short. On glass the germ tubes are usually longer and though appressoria are often formed within twenty-four hours, it is not unusual for them to develop during the second day: sometimes the germ tubes grow to considerable length and branch without forming appressoria. The spores germinate well and form appressoria on leaves other than tea (e.g. *Eucalyptus*), though tea is the only known host of this parasite. It is evident that no specific exudate from tea leaves or from leaves in general is essential for appressoria production, or they would never be formed in distilled water on glass. A more important factor is the thickness of the water film, and this is controlled to a large extent by the wettability of the surface on which the spores are placed. Two large globules of water were placed a short distance apart on a spore print on a glass slide which was then placed in a damp chamber at room temperature. The following day some of the spores beneath the water spots, particularly those near the edges, had germinated but not formed appressoria, whereas the spores not originally wetted had germinated well and produced many appressoria. Evidently the last-named spores obtained free water either by condensation during the night or in some other way, because the spores will not germinate in the absence of free water. Air previously bubbled through water may be passed over spores on glass for several days without starting germination though the same spores germinate afterwards when supplied with a film of free water.

The mode of entry of *Exobasidium vexans* into tea leaves is similar to that of many other fungi with appressoria. At its point of contact with a tea leaf or young stem the appressorium puts out an extremely fine thread which forces its way through the cuticle and outer cell wall, and then on entry into the cell, immediately expands to normal hyphal thickness. As the mechanism of penetration is entirely physical the importance of firm attachment by the appressorium becomes evident. If, as is considered probable, the mucilage which coats the germ tube and appressorium is the adhesive, the quantity of water around them may be of importance.

Hyphae (penetration tubes) which enter epidermal cells do not branch in that layer. Further growth is almost entirely intercellular though short irregular-shaped haustoria penetrate the cells. Blister formation results from the enlargement but not division of the invaded cells, whose contents become disorganized. Discoloration does not occur as a rule until sporulation is complete, when the area blackens and later falls out. The hymenium is formed beneath the lower epidermis as dense tufts of hyphae, with their

bases between the cells of the second mesophyll layer. Many cells of the mesophyll in the layer immediately below the epidermis are either crushed or dislodged. The epidermis breaks and flakes off to expose the white sporulating surface. The hymenium stains deeply with many dyes (safranin, iron haematoxylin, thionin, etc.), but mycelium in other parts of the leaf does not stain well and is therefore more difficult to demonstrate. Haustoria are best seen in stem tissues and very early stages of leaf infections.

Each blister on a leaf results from a separate infection. When infections are extremely numerous, as when a spore print is obtained on a young leaf, no perceptible blisters are formed though the whole of the lower surface of the leaf may become white with numerous hymenia and spores. The area invaded from one point of infection is restricted and the fungus never penetrates the whole of an expanded leaf. The size of blisters varies considerably and is dependent on the age of the invaded leaf, weather conditions and possibly the number of other infections in the leaf.

Infections on young stems do not give rise to prominent blisters, though the enlarged cells and dense growth of hymenium increases the stem's diameter slightly at the infected zone. The mycelial growth within infected stems is often extensive, frequently reaching the pith. Stem invasions are most destructive as young stems are killed thereby.

Entry into a leaf may be achieved within eight hours of the spore being wetted, and the hymenial surface is developed and matures two or three weeks later.

The spores of *E. rhododendri*, which causes somewhat similar blisters on *Rhododendron* in Ceylon, germinate differently. In this species small secondary spores are produced either directly from the basidiospore or from short germ tubes arising from that spore. Such secondary spores multiply by budding. In none of our many experiments with *Exobasidium vexans* spores have secondary spores been seen.

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A MODIFICATION OF THE 'NECOL' TECHNIQUE FOR MOUNTING MICROFUNGI

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For a number of years mycologists have used 'Necol', a cellulose acetate preparation, for making mounts of microfungi in which mycelium and fructifications are borne on the surface of the substratum. The 'Necol' is thinned down with acetone until it flows like glycerine. A small drop of this fluid is placed on the fungus colony and allowed to dry; this generally takes 15–30 min. A thin, colourless film or 'flip' is formed with the fungus firmly embedded in it, and this, when quite dry, is eased off with a fine scalpel and mounted in pure glycerine, using the double-coverslip method.

A modification of this technique has proved of value in recent studies of *Clasterosporium caricinum* and other hyphomycetes. Instead of being mounted in glycerine, the dry 'Necol' flip is placed on a glass slide and acetone is pipetted on to it. The cellulose acetate film dissolves and spreads out in a ring leaving the fungus behind; this ring is wiped off with a cloth. Repeated gentle washing with acetone, using a fine bulb pipette, clears away practically all the 'Necol'. A drop of lacto-phenol (or lactic acid) with cotton blue is then placed on the fungus, a $\frac{5}{8}$ in. square coverslip attached, and the preparation gently warmed.

With developing dematiaceous hyphomycetes hyphal tips and young conidiophores and conidia stain readily. When very dark older material is to be examined it is frequently an advantage to omit the cotton blue.

Lacto-phenol and lactic acid mounts are easily sealed with 'Noyer' and 'Krönig' cements, or better still, with an excellent cement, recently devised by Mr S. Waller, composed of equal parts of pure beeswax and dammar. When sealing, the slide outside the coverslip and the coverslip itself should be quite dry. It is never good policy to put on too much lacto-phenol in the first instance; if necessary, more can always be run in under the coverslip later. Cements are applied with a heated sealing implement (a length of thick brass wire with the last half inch bent at right-angles may be used). The cement is run evenly around the edge of the coverslip and then squared off neatly with the still-warm sealing tool.

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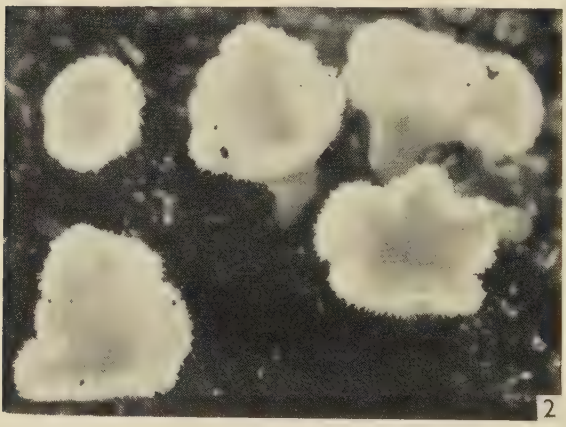
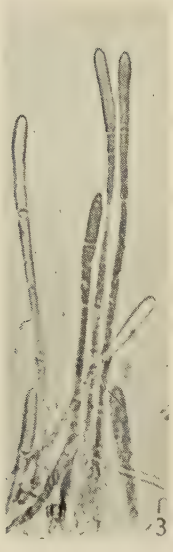
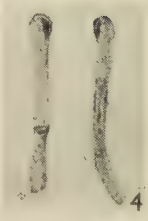


Figs. 1 and 2. Living *Dasyscypha nivea* (Grad. no. 394). ×15.





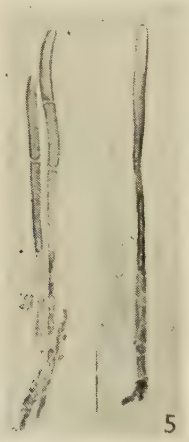


Fig. 3. Hairs of the above. ×630. Fig. 4. Hairs of Cooke, *Fungi Brit.* **1**, 559. ×630.
Fig. 5. Hairs of Carleton Rea specimen s.n. *Dasyscypha crystallina*, Perth, June 1920. ×630.

NOTE ON TWO *DASYSCYPHA* SPECIES

By W. D. GRADDON, B.Sc.

(With Plate 2)

Adequate descriptions of many of the small white *Dasyscypha* species are lacking in our literature, and the finding of a particularly fine colony of *Dasyscypha nivea* sensu Cooke has afforded the opportunity to expand the rather slender description given by Phillips (1893). In particular, the hairs—typical of the Niveae group—have been photographed as also have those of Cooke, *Fungi Brit.* **1**, 559, for comparison. Rehm (1896) regarded the latter as an example of his *Lachnum crystallinum*, but examination of Rehm Ascom. 14 shows this to have strongly lanceolate paraphyses greatly exceeding the asci. The material described by Carleton Rea (1920) as *Dasyscypha crystallina* is certainly wrongly named; it has relatively short hairs which enlarge apically to a subglobose head and which much resemble those of *D. spiraeaecola* (Karst.) Sacc. It would appear that *L. crystallinum* Rehm yet remains to be recorded for Britain.

My thanks are due to Dr Ramsbottom of the British Museum for facilities given to examine the relevant exsiccati.

Dasyscypha nivea (Hedw. ex Fr.) Sacc. *Syll. Fung.* **8**, 437 (1889)

Apothecia closely gregarious, shining white or slightly yellowish, conspicuously hairy all over; somewhat funnel-shaped at maturity, the stem enlarging upwards and passing gradually into the outer wall of the cup; cups to 3 mm. across with the margin sometimes intricately crenulate. On drying, brown and much shrunken. Hairs 80–110 μ long; about 3 μ across at the base, gradually attenuating upwards and then enlarging to an elongate subcylindrical head 15–20 μ long by 3–4 μ across; filament thin-walled, 4- to 5-septate, the lower part heavily encrusted with small crystals; the head is smooth, has a somewhat solid appearance and is not infrequently divided by a pronounced septum across its lower part; apical crystals were not seen, but the hairs are thickly interspersed with irregularly shaped rounded brown lumps which often bind them together in clusters. Asci 45–50 × 5 μ, 8-spored, the pore blued by iodine. Spores 6–8 × 2 μ, hyaline, continuous, eguttulate, with ends somewhat acutely narrowed. Paraphyses to 60μ long and thus not greatly exceeding the asci; very slenderly lanceolate, not exceeding 2·5 μ at the base of the elongate lance-head; filament 1- or 2-septate.

On a dead oak stump at Congleton, Cheshire; December 1947.

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A STUDY OF *MYCENA SANGUINOLENTA*

By E. MARGARET E. FORSYTH
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(With Plate 3 and 2 Text-figures)

Introduction

Mycena sanguinolenta is described by Fries (1821) in his *Systema Mycologicum* as *Agaricus sanguinolentus* and in the *Icones* of Fries (1867–84) the generic name *Mycena* is introduced. The specific name *sanguinolenta* refers to the blood-like fluid which exudes from the base of the stipe when the fructification is plucked and probably to the reddish brown colour of the pileus. Rea (1922) gives a general description of the features of the sporophore. The only investigations carried out on this species, of which records are available, are those of Bothe (Buller, 1934). Bothe found that some specimens had luminous gills and believed that the luminosity was due to something derived from the decayed leaves on which the fungus was growing.

Mycena sanguinolenta was found growing in a birch-heather association at Stravithie near St Andrews. The fructifications were found from early July to mid-October. Fries (1821) gives August to October, and Rea (1922) May to December as the time of their appearance. It was isolated in pure culture by Dr J. A. Macdonald in July 1946. Fresh sporophores were collected in the same locality by me in October 1946, and by Dr Macdonald in July 1947.

The fungus was identified from these sporophores by Dr Ramsbottom.

Methods

(*a*) *Fixation*

Mycelium grown by the coverslip technique (Macdonald, 1947) was fixed in Blès fluid. The formalin in this fixative reacts with the gelatin in the film forming a pectic substance and thereby causing the film to adhere to the coverslip. Using a second method the coverslips were dipped in Carnoy's fixative for 30 sec., washed in distilled water and fixed in Belling's modification of Navashin's fluid (La Cour, 1937, 1947). This method caused distortion and bursting of hyphae.

Sporophores were fixed in Blès fluid or half strength Calberla's fluid (Gwynne-Vaughan & Barnes, 1937).

(*b*) *Embedding*

First Rawlins's (1933) alcohol-cedar wood oil-paraffin wax schedule was followed, but with three changes of paraffin wax to remove the cedar wood oil completely. Paraffin wax (m.p. 49° C.) with ceresin was used. Later tertiary butyl alcohol was substituted for cedar wood oil (Johansen, 1940). This reduced shrinkage of tissues.

(*c*) *Sectioning*

Microtome sections were cut from 2 to 20 μ thick. Sections 14 μ thick gave the best results. Ribbons were fixed to the slides with Hewer's (1937) solution.

(*d*) *Staining*

Fresh material of gills and spores and material fixed in Blès fluid were stained in cotton blue in lactophenol (Rawlins, 1933) and mounted in glycerine jelly.

Mycelia grown on coverslips were stained with: (i) Nile Blue in 70 % alcohol (McLean & Cook, 1941). (ii) 0·5 % Heidenhain's haematoxylin (McLean & Cook, 1941). The films were hydrolysed for five minutes at 60° C. in normal hydrochloric acid before staining. (iii) Feulgen staining (La Cour, 1937, 1947). This last gave preparations which, owing to the delicate nature of material and technique alike, were too translucent for accurate observations.

Embedded material, if fixed in Flemming's fluid, was bleached with hydrogen peroxide before staining (Gwynne-Vaughan & Barnes, 1937). It was stained with: (i) Flemming's triple stain. (ii) 0·5 % Heidenhain's iron alum haematoxylin followed by light green or orange G in clove oil. (iii) 0·5 % Heidenhain's iron alum haematoxylin after hydrolysis at 60° C. with normal hydrochloric acid. (iv) Potassium permanganate. (v) Chlorazol Black E (McLean & Cook, 1941). (vi) Feulgen stain alone and with light green (La Cour, 1937, 1947). Really good results were obtained only with method (iii).

(*e*) *Vital staining*

Living mycelium was stained with Janus green (Guilliermond, 1941). The general protoplasm stained green, while the vacuoles were seen as mauvish pink globules. Nuclei were not revealed.

(*f*) *Cultures*

Mycelium was grown on the following media: (i) 2 % Japanese agar containing 2½ % malt (Rawlins, 1933). (ii) 2 % British agar containing 2½ % malt. (iii) Sawdust tubes with accelerator added (Badcock, 1941); the ash used was from heather wood. (iv) Sterilized or unsterilized *Sphagnum*, dead birch leaves and dead heather mixed together. (v) Sass (1929) culture medium on coverslips (Macdonald, 1947). I further modified the method by substituting for the brass frame, nickel foil cut to fit $2 \times \frac{7}{8}$ in. coverslips which were less cumbersome to handle. Hot Sass medium was poured on to cold coverslips, gentle manipulation ensuring the even distribution of a very thin film over the glass surface. This is more easily dehydrated than a thick film, and the changes in hyphal orientation are minimized, thus making microscopic examination more critical. Living hyphae can be studied under the microscope over long periods of time.

Description of the fungus

(*a*) *Sporophore*

(i) *General*

The fresh stalk is normally 5–7 cm. long, by 1–2 mm. in diameter. These figures agree with those given by Stevenson (1886) and Rea (1922). The stipe may elongate to 12·5 cm. when grown in *Sphagnum* (Stevenson, 1886). The stipe is reddish brown, flaccid, weak, glaucous except at the base where there are a number of rhizoid-like 'hairs' which disappear into the neighbouring medium (Pl. 3). It is composed of tightly packed hyphae running longitudinally and forming a hollow cylinder. The four quadrants of a stipe, cut from the base upwards, curve outwards, showing the presence of longitudinal tensions in the hyphae (Buller, 1909). The diameter of the stipe hyphae towards the inside averages 10 μ, those on the outside 5 μ. The walls are thicker in the narrow peripheral hyphae, thus the tension is greater in them than in the inner hyphae, causing outward curvature when the stipe is quadrisected.

It was noted by Buller (1909) that the stipe of species of *Mycena* may undergo geotropic curvature. The writer has also noted in *M. sanguinolenta* that the sporophore is positively phototropic (Pl. 3). A blood-red milk-like juice is extruded from the base of the stipe when broken.

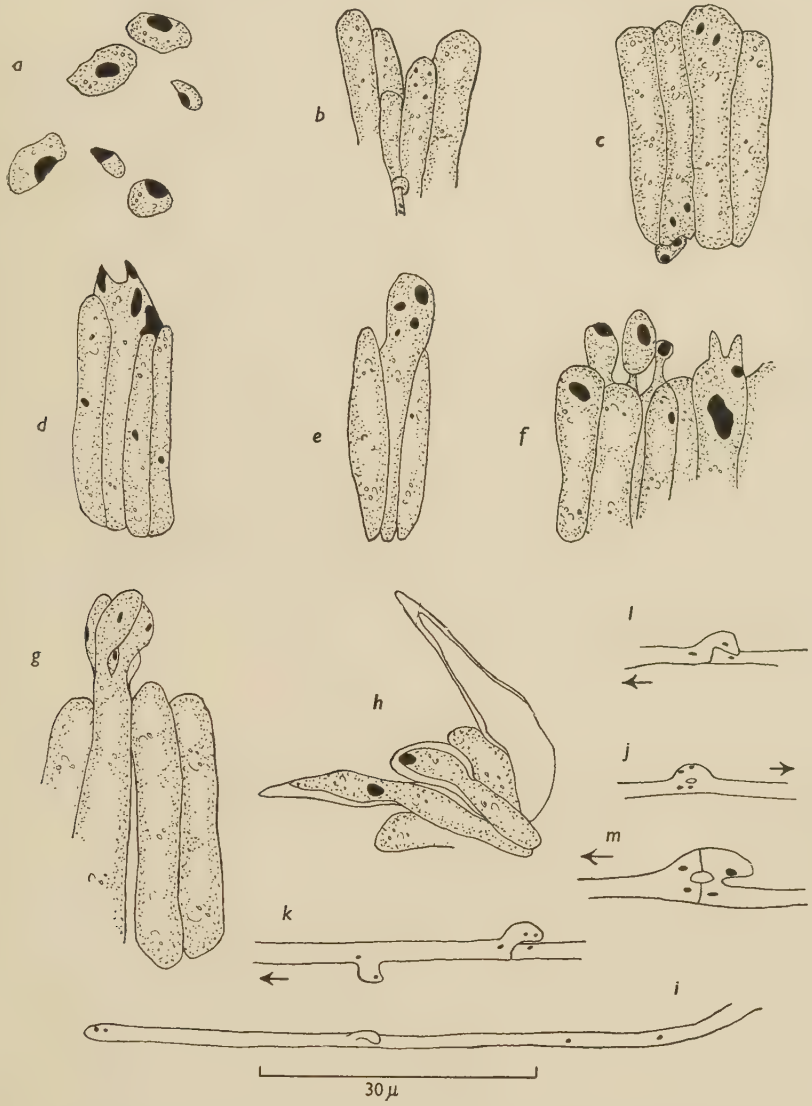
The pileus is bell-shaped, pale reddish brown with darker longitudinal stripes which merge together to give a uniform colour to the upper more steeply conical region (Pl. 3).

The pilei average 6 mm. in diameter. Rea (1922) gives a range from 4 to 20 mm. The gills are adnate to the top of the stipe, each alternate one being shorter than the pileus. They are pale membranaceous and edged with purple. The fructifications do not resist desiccation.

(ii) *Microscopic*

Buller (1909) found with species of *Mycena* that spores were shot about 1 mm. in the horizontal plane from the sterigmata to enable them to fall clear of the gills. This experiment was carried out by the writer. Thin transverse slices of the fresh pileus were placed under the microscope and the spores were seen to be projected in this way. Spores range in size from 4·5 × 3·5 to 9·0 × 5·0 μ, averaging 6·5 × 4·5 μ (Text-fig. 1 *a*).

Rea (1922) gives 8·9 × 4·6 μ. Differences in mounting fluid used probably account for this discrepancy. Fresh spores mounted in cotton blue and lactophenol averaged 6·9 × 4·8 μ, those fixed first in Blès fluid 6·1 × 4·4 μ. Only a few of the largest spores approximated to the sizes given by Rea. The gills are covered by the fertile hymenium composed of the ends of hyphae turned at right angles to the surface of the gill. These portions form the basidia or paraphyses. According to Maire (Buller, 1922) the basidia of *Mycena*, in common with those of *Psalliota*, *Godrina*, *Cantharellus*, *Clavaria*, *Geaster*, etc., produce either two, three, five or six spores in a single generation, or two generations each of four spores, or two generations with unequal numbers of spores, the same sterigmata being used for successive generations. The writer has found no evidence of this type of spore pro-



Text-fig. 1. *Mycena sanguinolenta*. *a*, basidiospores. *b*, conjugate nuclei approaching clamp at base of young basidium, four nuclei in older basidium. *c*, clamp at base of basidium after division of conjugate nuclei. Older basidium after first division of fusion nucleus. *d*, three young basidia with fusion nuclei. Older basidium with four nuclei, two of which have entered sterigmata. *e*, basidium with four nuclei protruding beyond neighbouring basidia. *f*, basidia bearing basidiospores, young basidiospore forming at tip of sterigma. *g*, basidium bearing four basidiospores. *h*, section of hymenium showing empty cystidium and cystidium with protoplasmic contents. *i*, tip of vegetative hypha with clamp connexion and showing distribution of nuclei. *j*, clamp connexion after division of conjugate nuclei. *k*, budding clamp connexion one of conjugate nuclei has moved into clamp also budding clamp in which both nuclei have divided and cross-wall has formed in main hypha. *l*, budding clamp, nucleus in main hypha has divided and septum has formed. *m*, 'Noble's' clamp connexion showing four nuclei and septae.

duction in *Mycena sanguinolenta*. The phenomena associated with spore production are as follows: (i) young clamp connexions appear associated with two nuclei in the parent hypha (Text-fig. 1 *b*), (ii) The completed fused clamp with two nuclei in the parent hypha and two in the clamp connexion (Text-fig. 1 *c*). (iii) One from each pair of nuclei presumably migrates into the base of the basidium where fusion takes place (Text-fig. 1 *d*). (iv) The basidium increases in size. The fusion nucleus moves towards the apex of the basidium. (v) The nucleus divides twice to give four spore nuclei which congregate at the upper end of the basidium (Text-fig. 1 *b*, *c*). (vi) The basidium projects beyond the surrounding basidia and its apex becomes swollen (Text-fig 1 *e*). (vii) Four conical sterigmata are pushed out from the end of the basidium in such a way as to form the four upright corners of a cube. (viii) A single nucleus passes into each of the sterigmata (Text-fig. 1 *d*,*f*). (ix) Cytoplasm collects round the nucleus, thus a basidiospore is formed at the end of the sterigma (Text-fig. 1*f*) from which the basidiospore is projected when ripe (Text-fig 1 *a*, *f* and *g*).

The ripe spores are shaped like orange pips with the small beak at the point of attachment to the sterigmata. Basidia do not ripen simultaneously throughout the gill. Cystidia occur interspersed among the basidia particularly at the gill tip and the point of attachment of the gill to the stipe. They have bulbous bases among the basidia and thorn or cone-like apices projecting from the hymenial layer (Text-fig. 1 *h*). Their rigidity helps to prevent crushing of the basidia. They are thick walled and may have protoplasmic contents.

(*b*) *Cultures*

The 7 cm. surface of a malt agar slant was covered in four weeks at 22° C. in darkness. Cultures kept at 14° C. grew at about $\frac{2}{3}$ this rate. Growth stopped in tubes buried in snow for one week. Normal growth rate was resumed when the tubes were brought back to 22° C. Light did not affect the rate of growth or the character of the mycelium.

In some malt agar-tube cultures zones of denser growth were observed. These corresponded with regions of freer branching in the mycelium. These periods of more active growth could not be correlated with any particular environmental condition. Similar rings have been observed in cultures of *Typhula gyrans* (Macdonald, 1934). Dense white specks consisting of much branched hyphae occur in many cultures. No specialized structures were observed.

Inoculation on Badcock's (1941) wood sawdust medium failed to induce the fungus to produce sporophores. A cotton-wool like mycelium was produced, the same type of growth was observed on sterilized 'natural' medium. Fructifications were produced only on this medium and only when it was unsterilized. The mycelium in this case was yellowish and inconspicuous. Possibly the production of fructifications was due to the stimulation by competition with other fungi and possibly due to the presence of organic or inorganic substances which are changed on sterilizing.

(*c*) *Coverslip film mycelia*

(i) *Nuclear phenomena in the mycelium*

The nuclei occur in pairs in the hyphae, the components of each pair are usually from 3 to 5 μ apart, while each pair is usually a distance of 30 μ from the next (Text-fig. 1*i*).

Conjugate pairs of nuclei are found in the tips of 10 % of the hyphae. Nuclei have also been seen 5–10 μ from the tips. Nuclei move freely in the hyphae, and it seems likely that there is frequent movement to the tip and back by nuclei normally situated some distance behind the tip.

(ii) *Clamp connexions*

Both Bensaude's types of clamp connexion (Buller 1933) are formed in *Mycena sanguinolenta*. That in which the septa are formed after the hook has fused with the parent hypha is much commoner than that in which the formation of the septa precedes fusion (Text-fig. 1*j–l*). The third type described by Noble (1937) for *Typhula trifolii*, in which the clamp is completed by the growth of a bridging hypha between hook and main hypha, has also been noted, but is rare (Text-fig. 1*m*).

Clamp connexions are formed freely, usually 30–40 μ behind the tip of the hypha (Text-fig. 1*i*). The hook always grows away from the tip. Usually the wall of the main hypha is depressed by the tip of the hook before the walls break down and the cytoplasm of the hook and hypha become confluent, indicating the exertion of a definite physical pressure on the hypha by the hook.

No normal, and only occasional abortive clamp connexions, show the associated peg on the main hypha which Buller (1933) described as a regular occurrence in *Coprinus sterquilinus* and *C. lagopus*.

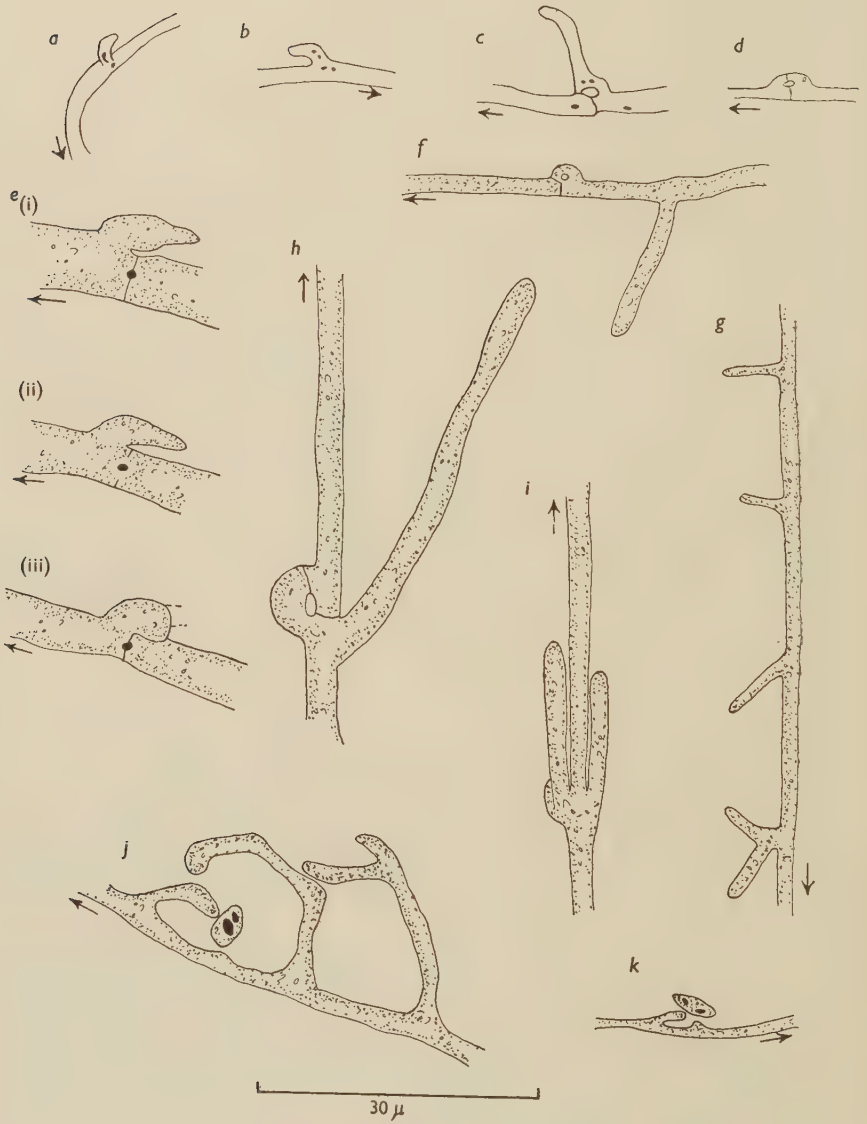
In the few examples observed the hook grows so far and stops. A small bulge then appears on the main hypha but union fails to take place. These structures were found only when the hyphae were growing directly on the damp coverslip beyond the nutrient medium. The failure to fuse may be a starvation effect.

The following nuclear phenomena were seen. The conjugate nuclei separate. One remains in the hypha and the other moves into the clamp. (Text-figs. 1*k*, 2*a*). This usually occurs while the clamp is still at the hook stage. Both nuclei divide (Text-fig. 2*b*). Generally the nucleus in the parent hypha divides first, while that in the clamp lags behind. The nucleus in the clamp has been seen to divide either in the incomplete hook or in the completed clamp (Text-figs. 1*k*, 2*b*).

After division is completed a cross-wall forms in the parent hypha separating the two daughter nuclei (Text-figs. 1*k–m*, 2*c*)

Text-figs. 1*j*, *m* and 2*c* either show or indicate the formation of the septum after completion of the clamp, indicating the presence of the two types of clamp connexions described by Bensaude. The cross-wall also appears in the incompleted hook or, later, in the completed clamp separating its two nuclei (Text-figs. 1*m*, 2*d*).

During the whole process of clamp connexion formation the nuclei



Text-fig. 2. *Mycena sanguinolenta*. *a*, budding clamp connexion with nucleus just moving into clamp. *b*, budding clamp with four nuclei. *c*, completed clamp bearing small branch. *d*, completed clamp connexion. *e* (i), top focus, complete septum with nucleus in central position; (ii), middle focus, nucleus as before, two flanges of septum visible; (iii), bottom focus, reappearance of complete septum. *f*, part of vegetative hypha showing completed clamp and an independent branch. *g*, part of hypha bearing short branches found on scanty substratum. *h*, branch arising opposite clamp connexion. *i*, branches arising from, and opposite to, clamp connexion. *j*, binucleate conidium arising from convoluted hypha. *k*, binucleate conidium arising from what appears to be a ruptured clamp connexion.

appear in a state of extreme mobility, often shooting out of the field of vision and later returning, when they are not actively engaged in division.

Buller (1933) states that pores are permanently present in septa so that there is protoplasmic continuity between cells. Text-fig. 2*e* illustrates a pore. Text-figs. 2*e* (i), (ii) and (iii) show the same clamp connexion, bearing a small branch, at different depths of focus. In (i), top focus, the whole septum is shown with a nucleus protruding on either side of it; (ii), middle focus, shows the same nucleus centrally placed with the two flanges of the septum protruding from the wall of the hypha on either side of it; (iii), bottom focus, shows the reappearance of the complete septum. It is believed that the pore is present in all young septa but it has not been possible to ascertain if this is the case in more mature parts of the mycelium.

It was noted that clamp connexions occurred more frequently if the culture had become accidentally infected by a species of *Penicillium*. The presence of this fungus appears to inhibit the forward growth of the hyphae, instead nuclear activity is stimulated resulting in many clamp connexions with small branches arising from them. A dense mycelium is formed which rapidly becomes swamped by the invading fungus.

Whatever may be the phylogeny of clamp connexions, or whether or not they may be regarded as homologous with the ascus hook (Smith, 1938), it is clear that their occurrence may facilitate nuclear division by the provision of favourable spacial conditions; and also, in a heterothallic fungus like *Mycena sanguinolenta*, such structures may simplify the mechanism for the separation and segregation of the daughter conjugate nuclei which is essential for the maintenance of identical nuclear pairs.

(iii) *Branching*

After the clamp connexion is completed, branches may grow out in association with it. It must be emphasized that the production of branches does not necessarily occur, and isolated clamp connexions may be found on the hyphae. It is possible that this condition has given rise to the theory that septa are present in the mycelium of basidiomycetes without associated clamps (Kuhner, 1946). It has been found in *M. sanguinolenta* that septa do not occur independently, but are invariably found with clamp connexions. What appear to be independent septa in the main hyphae are always found to be in combination with completed or developing clamp connexions which may be partially obscured by overlying hyphae and are revealed only by careful focusing.

Where branches arise independently of clamps, cross-walls have not been seen separating them from the parent hyphae. These branches are usually at an angle of 45° to the main hypha and grow away from the point of inoculation (Text-fig. 2*f*).

Mycelium growing on a very thin film tends to produce many short, often bifurcated, branches growing at right-angles to the main hypha. Frequently these branches are produced on one side of the hypha only. They are not found in association with clamp connexions and occur only at the edge of the film, or on the coverslip beyond the film (Text-fig. 2*g*).

Probably branches of this type are produced in an effort to find sufficient food material, as only normal branching is found where this is abundant.

There are three possible ways in which branches may be formed in association with clamp connexions: (*a*) opposite the clamp (Text-fig. 2*h*); (*b*) from the clamp (Text-fig. 2*c*); (*c*) a single branch arising from the clamp with a second branch arising opposite to it (Text-fig. 2*i*).

An examination of eighty-six branches growing in connexion with clamps showed that eighteen were of type (*a*), thirty-one of type (*b*), and thirty-seven of type (*c*). They may also grow parallel, and close to, the main hypha, but this type of branching occurs less frequently than the former (Text-fig. 2*i*).

If a single branch arises from the clamp it commences to grow almost immediately after the clamp is completed. The two nuclei in the clamp move into position just below the budding branch and later pass into it. In the case of a branch arising opposite the clamp connexion there is a time lag of about one-quarter of an hour after completion of the clamp, before the branch commences growth. If a second branch arises from the clamp it makes its appearance about five to ten minutes after the first branch swelling becomes visible. Thirty-five minutes after completion of the clamp the two young branches are quite distinct. It was not possible to discern the position of the nuclei relative to these young branches. The nuclei were seen to be in a state of agitation, and in one instance the pair from the clamp were observed passing through the septum, and both pairs could then be seen in the parent hypha on the side of the septum nearer the tip.

(iv) *Anastomosis*

Anastomosis rarely takes place in the mycelium. Occasionally two hyphae lying parallel to each other become connected by means of a small bridge.

(v) *Conidia*

Kuhner (1946) quotes Kniep as giving the name conidia to bodies which become detached from the mycelium and serve to disseminate the fungus. Bodies answering to this description have been seen in *M. sanguinolenta*.

At the onset of unfavourable conditions, for example, lack of nutriment, the mycelium gives rise to an abnormal number of unusual-shaped branches which are independent of clamps. Binucleate conidia are produced at the ends of these branches. They are similar in size and shape to the basidiospores (Text-fig. 2*j*). Binucleate conidia have also been observed arising from what appear to be ruptured clamp connexions (Text-fig. 2*k*). These conidia have been seen to germinate.

Discussion and conclusion

An examination of the nuclei in the basidium shows that Maire's statement (Buller, 1922) that successive generations of spores are born on the sterigmata of *Mycena* does not hold good for *M. sanguinolenta*. Only one fusion nucleus was present in the basidium which on division never gave rise to more than four spores. After spore dispersal both the basidium and its sterigmata were seen to be devoid of nuclei.

Three kinds of clamp connexions were distinguished, two as described by Buller (1933), the third by Noble (1937).

Buller (1933) gives an account of the division of conjugate nuclei as described by Bensaude. In this the two nuclei are said to divide simultaneously. In *M. sanguinolenta*, however, there was often a slight time lag between division of the nucleus in the hypha and division of the nucleus in the clamp, the latter dividing either before or after completion of the clamp.

Kuhner (1946) states that cross-walls are present in the hyphae of basidiomycetes without being associated with clamp connexions. In *M. sanguinolenta* cross-walls were found only in association with clamp connexions.

According to Buller (1933), a small peg forms on the hypha which unites with the hook of the clamp, so completing the connexion. In *M. sanguinolenta* a peg is formed only in clamps which for some reason remain uncompleted. Where clamp formation is normal the reverse effect is seen, the main hypha becoming depressed before uniting with the hook.

Buller (1933) also states that there are permanent pores present in the septa of basidiomycetes giving protoplasmic continuity between the cells. These are present in *M. sanguinolenta*, the pores are sufficiently elastic to allow nuclei to pass from cell to cell. In older parts of the mycelium, where protoplasmic activity is at a reduced minimum, it was not possible to verify the presence of pores.

Paired nuclei have in a few instances been recorded at the tips of the hyphae, and at distances of 5 and 10 μ from the tip. Their normal situation is 30–40 μ behind the tip. It is thought that owing to metabolic activity the mobility of the nuclei increases and movement to and from the tip takes place.

The many small branches growing at right-angles to the main hypha, which are found when the substratum is scanty, do not appear to have been generally recorded. No nuclei have been seen connected with these branches, and it is thought that they arise unassociated with nuclear division.

If the culture medium becomes infected by a species of *Penicillium*, forward growth of the hyphae towards the infection is inhibited. Activity becomes centred on the clamp connexions, and a much branched stunted mycelium results which becomes swamped by the infection.

Summary

1. Fructifications of *Mycena sanguinolenta* were obtained from a birch-heather association near St Andrews.

2. The methods of treatment of this material and of mycelia grown, in pure culture, on coverslips are described.

3. A description is given of the general structure and microscopic detail of the sporophore. Spore dimensions and nuclear phenomena in the basidium are described.

4. Culture characters are noted. The nuclear phenomena occurring in the mycelium are detailed, particularly those associated with clamp connexion formation.

5. Three types of clamp connexion were found. Pores in septa were observed.

6. Types of branches formed independently, or associated with clamp connexions, are noted.
7. Anastomosing hyphae occur rarely.
8. Binucleate conidia are formed.

The writer wishes to thank the Professors of Botany of St Andrews University and University College, Swansea, for facilities placed at her disposal, and encouragement received. Her thanks are due to Dr Ramsbottom for identifying the fungus under investigation, and to Dr J. A. Macdonald who suggested the problem, supervised the work and was a source of encouragement throughout.

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EXPLANATION OF PLATE 3

Fructification with stipe showing phototropic curvature and bearing hairs at its base.
Fructification showing striped pileus.

(*Accepted for publication* 20 *October* 1948)

Source of
light

PRESERVATION OF YEASTS AND FUNGI BY DESICCATION

By MABEL RHODES
National Collection of Type Cultures, Elstree, Herts

(With 4 Text-figures)

Bacteria, in the form of dried cultures, are known to survive for long periods (Hammer, 1911; Rogers, 1914; Swift, 1921; Flosdorf & Mudd, 1938 and Stamp, 1947); morphological, physiological and antigenic characters, and virulence are retained in their original state, and degenerative changes associated with repeated subcultures are halted. A simple method of drying cultures was described to us by Prof. A. Sordelli, of Buenos Aires, and we have used it in the National Collection of Type Cultures since 1934. The viability of bacteria preserved by this method will be reported separately; the present paper gives the results obtained with cultures of fungi and yeasts up to the time the National Collection of Type Cultures stopped keeping these organisms.

Method

Cultures for drying were grown on a solid medium; non-sporing organisms were dried as soon as good growth developed, and incubation of sporing genera was continued until spores were produced. A loopful of culture or a small piece of mycelium, where possible with spores, was rubbed up in a loopful of sterile horse serum on the inner wall of a plugged sterile test-tube about 8 × 60 mm. (Fig. 1). This tube was placed inside a slightly wider tube (10 × 150 mm.) at the bottom of which was a small amount of phosphorus pentoxide (Fig. 2). The outer tube was then heated above the level of the inner tube's wool plug and drawn out (Fig. 3). The outer tube was attached to a Cenco Hyvac pump for exhaustion and when the vacuum was satisfactory was sealed off at the constriction (Fig. 4).

Dried cultures were stored at room temperature in the dark. To recover the organisms the outer tube was opened and the inner tube removed. About 1 ml. of liquid medium was pipetted into the small tube; suitable media were beer wort for most yeasts and many fungi, and glucose broth for the others. The tube containing medium was incubated at the appropriate temperature until growth appeared.

Results

After intervals varying from one month to thirteen years dried cultures of sixty-one different genera were tested for viability. Two or three weeks might elapse before growth was apparent and tubes were kept for at least a month before being discarded as sterile. The time lag observed with dried cultures is not unusual and may occur when transplants are made from

cultures on ordinary nutrient medium. Table 1 summarizes the viabiiity in years but does not differentiate genera among the 405 isolates tested.

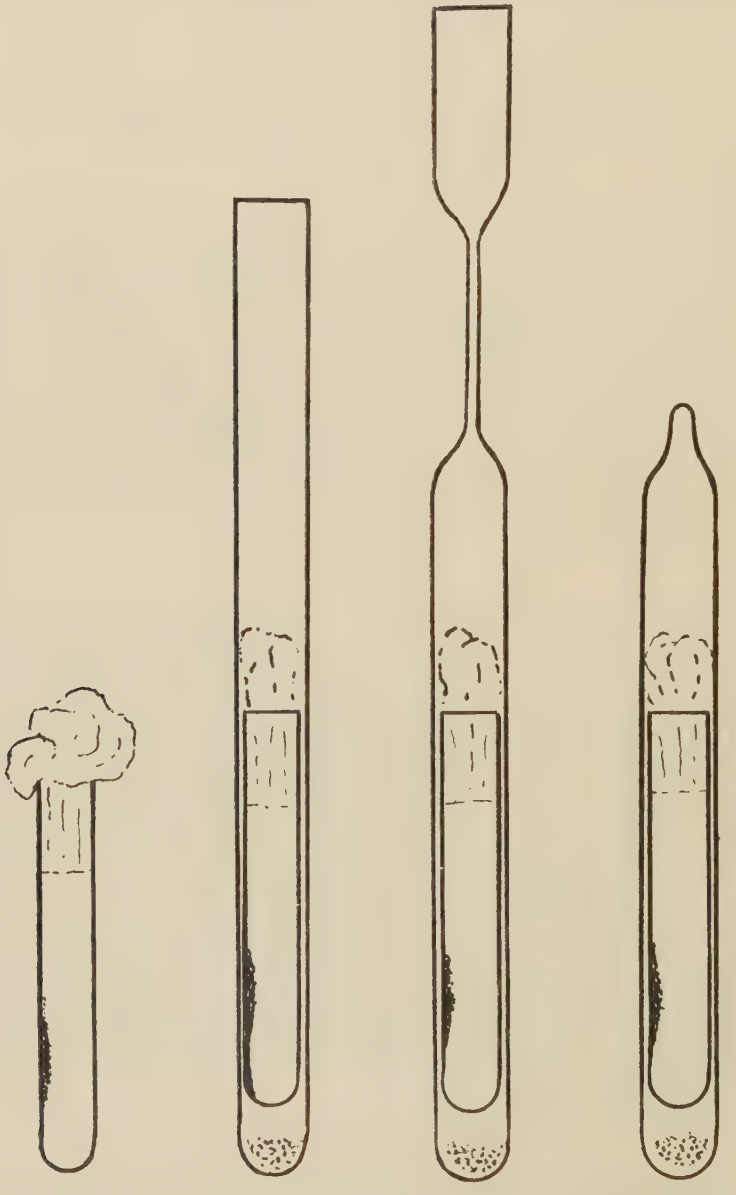

Fig. 1. Fig. 2. Fig. 3. Fig. 4.

There is little difference in the proportion of viable cultures at any time interval and for convenience in tabulating the results obtained with different genera, cultures opened within four years of drying are grouped

together, as are cultures tested between five and eight years, and between nine and thirteen years. A list of the genera dried and the number of viable cultures are given in Table 2.

Table 1. *Viability in years of dried cultures of fungi and yeasts*

Years between drying and test	No. tested	No. viable
Less than 1	8	6
1–2	9	8
2–3	22	22
3–4	11	5
4–5	7	5
5–6	16	11
6–7	9	8
7–8	119	108
8–9	154	136
9–10	121	112
10–11	87	74
11–12	4	4
12–13	7	5
13–14	10	9

Many genera were represented by a single isolate, but the value of the method can be seen in genera such as *Saccharomyces*, of which twenty-eight species were dried. On revival all cultures of *Aspergillus* spored well, and some genera spored better than the cultures of the same organisms that had been maintained for the same interval on the usual media.

Discussion

In the tests carried out several genera, such as *Acladium*, *Ashbya* and *Beauveria*, were represented by a single isolate and it cannot be assumed that other isolates or different species of these genera could be dried as successfully. However, the success achieved with many species of *Cryptococcus*, *Endomycopsis*, *Monilia*, *Saccharomyces*, and *Trichophyton* suggests that where a single isolate of a genus is capable of being dried other isolates or species of the same genus will behave in a similar way. From a much greater experience in the drying of bacteria we know that there are exceptions to a generalization of this sort. With the exception of *Aleurisma*, *Phytophthora*, *Sabouraudites*, *Schwanniomyces*, *Sphaceloma* and *Trichosporium*, in each of which only a single isolate was dried, all the genera tested were dried successfully.

Over the period of tests 87·9% of dried cultures were found to be viable; at the end of thirteen years the proportion of viable cultures was as great as the mean. Thus it appears likely that if a culture survives the drying process it will remain viable for a more or less indefinite period.

Summary

A simple technique, developed for preserving bacteria by drying, was tried on cultures of fungi and yeasts.

Nearly 88% of cultures tested survived for varying periods up to thirteen years.

Table 2. *Viability of different genera kept in dried state*

	No. of isolates	Time (in years) between drying and test: 1–4 No. tested	1–4 No. viable	5–8 No. tested	5–8 No. viable	9–13 No. tested	9–13 No. viable
Acladium castellanii	1	—	—	2	2	—	—
Aleurisma chartarum var. *orientale*	1	—	—	—	—	1	0
Ashbya gossypii	1	1	1	1	1	—	—
Aspergillus, 2 spp.	5	9	9	—	—	—	—
Atelosaccharomyces sp.	1	—	—	1	1	—	—
Beauveria cylindrospora	1	1	1	—	—	1	1
Bodinia violacea	2	—	—	5	5	—	—
Botrytis allii	1	—	—	—	—	1	1
Brettanomyces spp.	2	1	0	2	2	—	—
Candida, 6 spp.	7	—	—	9	9	3	3
Chalara mycoderma	1	—	—	1	1	—	—
Cladosporium mansonii	1	—	—	—	—	1	1
Coccidioides, 2 spp.	2	—	—	—	—	2	1
Cordyceps militaris	1	1	1	—	—	1	1
Cryptococcus, 11 *n.*, 7 *un.* spp.	21	—	—	18	15	16	15
Debaryomyces, 4 spp.	5	—	—	3	3	2	2
Endodermophyton, 2 spp.	4	—	—	3	1	2	2
Endomycopsis, 9 spp.	14	2	0	14	12	6	6
Epidermophyton, 3 spp.	3	—	—	2	2	3	2
Geotrichum, 8 spp.	11	—	—	8	4	7	7
Glenospora, 4 spp.	6	—	—	7	4	2	2
Hansenia apiculata	1	—	—	—	—	2	2
Hanseniospora valbyensis	1	—	—	—	—	1	1
Hansenula, 4 spp.	7	—	—	4	4	5	3
Hemispora stellata	2	—	—	4	4	5	3
Hormodendrum pedrosoi	2	—	—	2	2	2	2
Hyalodendron lignicola	3	—	—	—	—	6	6
Kloeckera, 15 spp.	16	—	—	2	2	21	10
Malassezia sp.	1	5	4	—	—	1	1
Micromonospora, 3 spp.	3	—	—	5	2	—	—
Microsporum, 2 spp.	2	—	—	4	2	—	—
Monilia, 19*n.*, 5*un.* spp.	35	3	3	22	22	33	33
Monosporium, 2 spp.	2	—	—	—	—	2	2
Mycoderma, 4 spp.	4	—	—	3	3	2	2
Mycotorula, 7 spp.	13	—	—	10	10	13	13
Nadsonia fulvescens	1	1	1	1	1	—	—
Nematospora coryli	5	—	—	9	9	—	—
Oidium, 3 spp.	6	2	2	5	4	2	2
Parasaccharomyces ashfordii	1	—	—	—	—	2	2
Phytophthora porri	1	1	0	—	—	—	—
Pichia, 8 spp.	9	—	—	5	5	7	7
Pityrosporum ovale	1	1	1	—	—	—	—
Prototheca, 2 spp.	2	—	—	4	4	—	—
Rhodotorula, 8 spp.	10	—	—	9	9	2	2
Sabouraudites felineus	1	1	0	1	0	—	—
Saccharomyces, 28 spp.	55	9	9	26	26	28	28
Saccharomycodes ludwigii	2	—	—	1	0	2	2
Saccharomycopsis capsularis	2	—	—	—	—	2	2
Schizosaccharomyces, 3 spp.	4	4	2	1	1	2	2
Schwanniomyces occidentalis	1	—	—	—	—	1	0
Sphaceloma violae	1	—	—	—	—	2	0
Sporendonema epizoum	1	—	—	2	2	—	—
Sporobolomyces roseus	1	—	—	2	2	—	—
Sporotrichum, 6 *n.*, 1*un.* spp.	13	—	—	13	13	9	9
Torula, 22 *n.*, 7 *un.* spp.	32	2	2	32	32	7	6
Torulopsis, 17 spp.	19	5	5	19	16	2	2
Trichophyton, 11 *n.*, 1*un.* spp.	12	3	2	11	3	7	6
Trichosporon beigeli	1	—	—	1	1	1	1
Trichosporium rugosum	1	—	—	1	0	—	—
Zygopichia, 2 spp.	2	—	—	1	1	1	1
Zygosaccharomyces, 7 spp.	8	2	1	8	8	2	2
Unclassified yeasts	30	3	2	14	13	14	13

n. = named species. *un.* = unnamed species

Dr R. St John-Brooks started drying cultures by the method outlined in this paper and it is to his interest and encouragement that I owe my thanks.

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AMPHICHAETA GREVILLEAE N.SP., ON *GREVILLEA ROBUSTA* SEEDLINGS

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(With 2 Text-figures)

A disease of young *Grevillea robusta* seedlings occurred in a nursery in the Kandapolla district (elevation 6500 ft.) in September 1947. The plants, many of which were completely defoliated, were 2–3 in. high, but such

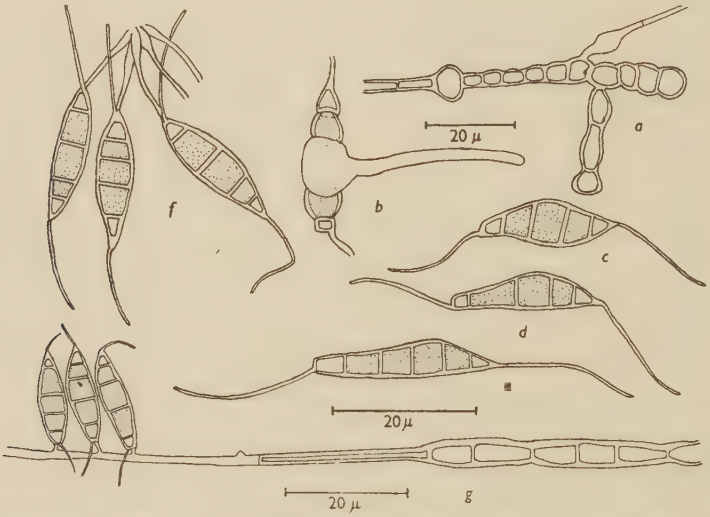

Fig. 1. *Amphichaeta grevilleae* n.sp. *a*, microsclerotia from culture; *b*, germinating conidium in hanging drop after sixteen hours; *c–e*, mature conidia from leaf; *f*, conidia and conidiophores from an acervulus on leaf; *g*, conidia produced in a young culture.

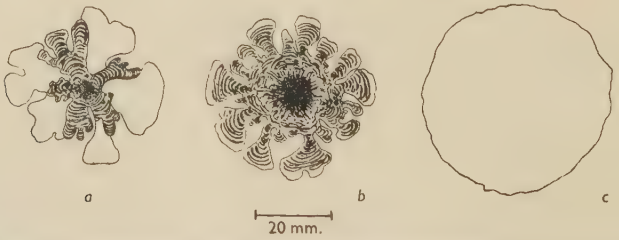

Fig. 2. *A. grevilleae*. *a*, original single spore isolation showing black and white sectors; *b*, subculture from black portion; *c*, subculture from white portion.

leaves as remained attached were severely blotched. The necrotic areas, which ranged in size from small specks to patches involving almost the whole leaf, bore black acervuli on both surfaces. Microscopic examination showed the fungus to be an undescribed species of *Amphichaeta*.

The spores germinate readily in water. They are fusiform, 4- or 5-septate, with dark coloured median and hyaline terminal cells. At each end is a single hyaline seta (Fig. 1 *c–e*). On germination the germ tube invariably arises from one of the median cells. In water the whole spore swells slightly, and the cell from which the germ tube will arise enlarges considerably. Usually a thick hyaline germ tube emerges from the enlarged cells within sixteen hours in hanging drops at room temperature (*c.* 20° C.) (Fig. 1 *b*). The germ tube elongates and branches but appressoria have not been seen.

Single spore cultures were easily obtained by plating spores direct from acervuli in Waksman's Agar without acid. Growth on this medium was slow, averaging about 2 mm. per day. At first the mycelium was white, but after a few days the centre of each colony became dark coloured. The extension of the dark area was very irregular as large lobes were formed (Fig. 2 *a*). Each colony then appeared to consist of two different fungi, one black and the other white. In the black area numerous micro-sclerotia (Fig. 1 *a*) were formed in the agar, and acervuli-bearing spores appeared on the surface. The white area, unlike the black, carried abundant aerial mycelium, but produced neither microsclerotia nor spores.

Six subcultures were made from the black zone and the same number from the white, all from different parts of one plate, using the same medium. The six cultures from the white areas gave similar white growth, with no spores or microsclerotia (Fig. 2 *c*) while subcultures from the black area gave black irregular-shaped growths and produced spores and microsclerotia (Fig. 2 *b*).

The black and white subcultures appeared so different that one could hardly realize that they were the same fungus. When the white fungus was subcultured to sterile *Grevillea* leaves spore pustules were formed which demonstrated its identity. The spores were identical with those produced by the black fungus.

As healthy *Grevillea* seedlings were not available at the time, young leaves from trees were used for inoculation experiments though the disease has not been observed on adult plants. Spores were used for inoculation and all attempts to cause the disease on unwounded leaves failed. On leaves wounded before inoculation, blotches developed similar to those seen on the seedlings. The fungus was recovered from these blotches.

The prevalence of the fungus on all the seedling leaves, coupled with the fact that similar necrotic lesions are caused by the same fungus on young wounded leaves of older plants, leaves little doubt that the fungus is pathogenic. As the species has not been described previously I propose the name *Amphichaeta grevilleae* for it.

Amphichaeta grevilleae n.sp.

Pustules black, scattered, sparse, erumpent, on both sides of diseased leaves. Conidia fusiform, 15·8–25 × 4·6–7 μ, 4–5-septate, not constricted, median cells coloured, terminal cells hyaline; apical and basal setae 13–21 μ long; basal seta inserted near side of the conidiophore. Conidiophores filiform, hyaline, 12·6–15 × 1·3 μ.

Habitat. On leaves of *Grevillea robusta* Kandapolla, Ceylon.

Pustulis nigris, non-gregariis, sparsis, erumptibus, amphigenis, conidiis fusiformibus, 15·8–25 × 4·6–7 μ, 4–5-septatis, non-constrictis, cellulis medianis coloratis, extremis hyalinis; setulis apicalibus basilaribusque 13–21 μ longis, basilari obliqui, prope basidii insertiones oriente; basidiis filiformibus, hyaline, 12·6–15 × 1·3 μ.

Hab. in foliis *Grevilleae robustae* Kandapolla, Ceylon.

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THE USE OF PLASTICS FOR MOUNTING THE LARGER FUNGI

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There have been many previous accounts of methods for mounting biological specimens in plastics. This report is published as a convenient record of the adoption and simplification of such a technique for mycological purposes.

The term 'plastic' covers a number of organic compounds which are liquids when in the monomeric state, and solids when polymerized. There are differences in the properties of the compounds, and selection must be made of the one most suitable for the material to be mounted. For most purposes it is advantageous to use one which polymerizes fairly quickly and which has a low shrinkage factor on solidification. The latter property is important, as the use of compounds with a high shrinkage factor is liable to involve both distortion of fragile specimens and the development of cracks or weaknesses in the finished block. Methyl methacrylate has been chosen as the most suitable cheap and readily available plastic in this country. The method of use is based on the polymerization of liquid monomer in which fungal material has been suspended, and it is carried out in the following stages:

(1) *Preparation of the monomer.* Commercial supplies usually contain a stabilizer to prevent polymerization during transit and storage. This must be removed, either by distillation or by the specific method given by the suppliers. In the case of methyl methacrylate supplied under the trade name 'Kallodoc', it is removed by washing two or three times with 5% NaOH. This washing may be carried out in a separating funnel, the NaOH being followed by distilled water until the washings are no longer alkaline. The monomer is then dried by standing in contact with anhydrous $CaCl_2$ for 24 hours, filtered, and stored in a glass stoppered jar at 4° C. until required.

(2) *Impregnation of the specimen.* Methyl methacrylate is not miscible with water but is miscible with alcohol, xylol, and a number of other organic solvents. If complete impregnation is desired it is therefore necessary to dehydrate and impregnate the specimen with successive alcohol and alcohol-monomer mixtures, the ratios and timings being dependent on the size and fragility of the material and on the need to avoid plasmolysis. The monomer is, however, tolerant to traces of water and while excess is likely to cause cloudiness or white discoloration of the finished block, many specimens can be embedded successfully without complete dehydration. Such specimens should be washed for a few minutes with 95% alcohol under reduced pressure, to remove the surface water film and the air from the tissues, then transferred direct to monomer. The natural appearance, opacity, and colour of the specimen is preserved most satisfactorily by this quick treatment, but there is risk of distortion of delicate tissues by plasmo-

lysis. The alternative of complete dehydration and impregnation has advantages for material mounted for anatomical study, as there is less chance of plasmolysis, and the bleaching and clearing action of the plastic produces a transparent preparation. The precise treatment required by any particular material must therefore be determined by experience and by the nature of the preparation required.

(3) *Polymerization of the monomer.* Polymerization takes place slowly at room temperature and can be accelerated by several methods, including heat, use of a catalyst, or by exposure to ultra-violet light. The rate of polymerization must be kept uniform throughout the medium, to ensure an even distribution of the shrinkage which occurs as it solidifies. If the solidification is not uniform tensions may be set up, resulting in the formation of cracks or bubbles in the finished block. Less severe tensions produce weaknesses in the block which may cause it to shatter like badly annealed glass during storage.

Uniform solidification is best obtained by maintaining a slow rate of reaction in the later stages of polymerization. In the early stages, while the plastic is still a free liquid, conditions can be kept uniform by constant shaking and a faster rate of reaction can be employed. A convenient method is to heat monomer containing a catalyst (0·1% benzoyl peroxide) at 85–95° C., under an air condenser until it becomes a thick syrup, shaking it constantly. The syrup should then be cooled to 35° C, and poured over the fungus material which has previously been suspended in, or impregnated with, liquid monomer. The specimen should then be held at 35–40° C., until it is solid, usually about eighteen to twenty-four hours. The reaction is exothermic and a water-bath is therefore needed to control the temperature during final solidification. If the temperature is allowed to rise at this stage there is risk not only of cracking due to uneven polymerization, but also of the volatile monomer boiling, the bubbles being caught in the viscous liquid and spoiling the appearance of the finished preparation. This boiling also occurs during the earlier quick polymerization process, and careful control is needed to ensure that the bubbles are removed by shaking and that the rate of reaction is slowed down by cooling before the medium becomes too viscous for use.

(4) *Cutting and polishing the solidified preparation.* When using glass containers for embedding, it has been found necessary to break the glass to remove the solid block. It may then be cut to shape with a hacksaw, its rough surfaces smoothed with successive grades of sand-paper or abrasive paste on a glass plate and finally polished with domestic metal polish on a soft duster. The ease with which the material can be cut and polished makes it possible to section specimens after embedding. This is particularly useful for elementary teaching, as preparations can be made to show the external characters on one side and details of structure on the cut and polished reverse.

I am indebted to Mr H. M. Hutchinson of the Plastics Division, The Distillers Company Ltd., Research Department, for suggesting the use of the technique and for much assistance during its development.

(*Accepted for publication* 12 *January* 1949)

STUDIES ON BRITISH PYRENOMYCETES

III. THE BRITISH SPECIES OF THE GENUS *DIATRYPELLA* CESATI & DE NOTARIS

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(With 5 Text-figures and 3 Graphs)

I. Introduction

(i) *The genus* Diatrypella

Some of the species now included in *Diatrypella* date from very early times. Fries (1823), in his genus *Sphaeria*, listed the following species:

Tribe VI, Lignosae, included
70. *S. aspera* Fr.
71. *S. favacea* Fr.
72. *S. verruciformis* Ehr.

Tribe VII, Versatiles, included
84. *S. quercina* Pers.

Tribe X, Incursae, included
155. *S. angulata* Fr.

Later Fries (1849) founded the genus *Diatrype*, to include the *Sphaeria* tribes Lignosae and Versatiles. Cesati & de Notaris (1863) proposed to restrict *Diatrype* to forms with cylindrical, usually curved, ascospores. To a section of this genus characterized by polysporous asci they gave the name *Diatrypella*. De Notaris (1863) raised *Diatrypella* to generic rank under the title of '*Diatrypella* Cesat. & de Not. Schem. sferiac. 28', and included three species as follows:

30. *Diatrypella favacea* Cesat. & de Not. Schem. sferiac. 28.
31. *Diatrypella Tocciaeana*.
32. *Diatrypella Rousselii* Cesat. & de Not. Schem. sferiac. 27. lapsu calami sub *Diatrype*.

(ii) *The British species of* Diatrypella

Massee (1887) published the following list of British species of *Diatrypella*, based on Cooke (1871) and certain later collections:

1. *D. verruciformis*, Ehr. Sacc. Syll. 743; Hdbk. 2432.
On branches. Common.
var. *affinis* Cke.
On elder. Whitehall. On alder. Shere.
2. *D. favacea*, Fr., Sacc. Syll. 745: Hdbk. 2431.
On *Betula alba*. Chislehurst, Lynn.
3. *D. nigro-annulata*, Grev., Sacc. Syll. 745; Hdbk. 2433.
(= *angulata*, Fr.).
On lime and beech. Scotland.
On oak and holly. Chislehurst, Weybridge, Eltham and Pentrick.

4. *D. Tocciaeana*, Not., Sacc. Syll. 747.
   On alder. Dinmore, Irstead, Lyme Regis.
5. *D. aspera*, Fr., Sacc. Syll. 753; Hdbk. 2430.
   On elm, beech, etc. Weybridge, Eltham, Burnt Ash Lane, Bishop's Wood, Lynn.
6. *D. quercina*, P., Sacc. Syll. 759; Hdbk. 2429.
   On oak. Common.

This list forms the starting-point of the investigation described in this paper which was carried out during the years 1936–9. After the experimental work was completed Bisby & Mason (1940) published a new list of British Pyrenomycetes. In addition to the species of *Diatrypella* listed by Massee, their list includes three other species, *D. discoidea* Cke. & Peck, *D. exigua* Wint. and *D. rhois* (Schwein.) Ell. & Ev. Unfortunately there has been no opportunity to carry out any work on these three species.

Up to the present time the naming of British specimens seems mainly to have followed the diagnoses given by Nitschke (1867), although certain later authors have doubted the validity of some of the species he described. Berlese (1905) suggested that *D. nigro-annulata* was a smaller form of *D. verruciformis* and that *D. favacea* and *D. tocciaeana* were scarcely separable. Von Höhnel (1915) declared that *D. nigro-annulata* and *D. verruciformis* were synonymous. One of the aims of this study is to show that of the six species of *Diatrypella* listed by Massee only *D. favacea* and *D. quercina* can be maintained as valid.

The second purpose of this investigation is to compare the development in culture of the conidia of the British species. Conidia associated with naturally occurring perithecial stromata have been described by Nitschke (1867), Tulasne (1863) and Fuisting (1867). Wehmeyer (1925) has described conidia of American species of *Diatrypella* in culture. It is intended to compare the conidia described by these authors with those produced in culture and those found associated with perithecia in British specimens.

II. Herbarium material of perithecial stromata compared with collected material on which culture studies are based

(1) *Diatrypella favacea* (Fr.) Ces. & de Not.

D. favacea, as the species is maintained in this study, includes a number of forms previously described as separate species. An authentic specimen containing ascospores was available of only one of these species, *Diatrype favacea* Fr., but it was possible to examine specimens of at least one of the exsiccati cited by Nitschke for each of the species *D. favacea*, *D. verruciformis*, *D. nigro-annulata*, *D. tocciaeana* and *D. aspera*. The characters of the stromata of each of these alleged species is discussed first because, as will be shown later, separation of the species depends almost entirely upon differences in the external appearance of the stromata.

Specimens of the exsiccati examined are cited below in the appropriate section. They are all in the Herbarium of the Royal Botanic Gardens, Kew.

(i) *Diatrypella favacea* (Fr.) Ces. & de Not.

Fries, *Scler. Suec.* No. 306.
Fuckel, *Fung. Rhen.* No. 1040.

The perithecial stromata in the Fries specimen are elliptical, transversely erumpent through the substratum periderm, 4–6 mm. long and 2–3 mm. broad. The surface of the stromata is free from the periderm, somewhat flattened, slightly roughened, brownish except for the black, six sulcate ostioles. The stromata in the Fuckel specimen are similar but are completely black and the ostioles are less distinctly sulcate. This specimen is stated to be on *Betula* and the Fries specimen appears to be on the same substratum.

Most of the British specimens on *Betula* which have been examined are very similar in form to these exsiccati. The colour of the surface of the stromata varies with their age; at first whitish, except for the black ostioles, and later brownish or almost black.

(ii) *Diatrypella verruciformis* (Ehr. ex Fr.) Nits.

Moug. & Nestl., *Stirp. Vog. rhen.* No. 867, on *Corylus*.
Klotzch, *Herb. Myc.* ed. II, No. 728.
Rabh., *Fung. Eur.* No. 135 (in Herb. M. C. Cooke).
Fuckel, *Fung. Rhen.* No. 1038 (sub.nom. *Diatrype ferruginea*).

In these specimens the stromata are black, cylindrical or angular, rather irregular, sometimes confluent, erumpent through the closely adhering periderm, 1·5–4 mm. in diameter. British specimens collected on *Corylus*, *Salix* and occasionally *Betula* resemble closely these exsiccati except that on *Betula* the stromata are almost invariably elliptical. On *Corylus* young stromata are frequently brown, later turning black.

Through the kindness of the Council of the Linnean Society I have been able to examine a specimen of *Sphaeria verruciformis* Ehr., *Crypt.* exs. 280, in the Smithian Herbarium which is housed in the Linnean Society's London rooms. The stromata of this specimen are of the type characteristic of *Diatrypella verruciformis* on *Betula* but no asci or ascospores could be found in the perithecia so that the specimen cannot be definitely identified as a *Diatrypella*.

(iii) *Diatrypella nigro-annulata* (Grev.) Nits.

Rabenhorst, *Fung. Eur.* No. 1822, on *Fagus*.

The stromata in this specimen are black, cylindrical, erumpent through the periderm, 3–5 mm. in diameter with black, three to five sulcate, ostioles. The black marginal line surrounding the stromata does not show clearly through the periderm in this specimen which is thus very similar to some specimens of *Diatrypella verruciformis*. British specimens on *Fagus* are of the *Diatrypella nigro-annulata* type. In some specimens the black line surrounding the stromata, this being the main distinctive character separating *D. nigro-annulata* from *D. verruciformis*, is clearly visible through the periderm but in others it cannot be seen.

(iv) *Diatrypella tocciaeana* de Not.

Rabenhorst, *Fung. Eur.* No. 253 (sub.nom. *Microstoma vulgare* Awld.). In Herb. M. C. Cooke.

Here the stromata are obtusely conical, often confluent and only slightly erumpent through the periderm. The ostioles are indistinctly three to five

sulcate or have a number of slight furrows. The British collections on *Alnus* are similar in appearance to this specimen.

(v) *D. aspera* (Fr.) Nits.

Fuckel, *Fung. Rhen.* No. 1036 (sub.nom. *Microstoma verruciforme* Awld. ad *Quercum*).

The stromata are small, cylindrical or angular, rather flat on top, erumpent through the periderm, with indistinctly sulcate black ostioles. Although the surface of each stroma is smoother than is usually found in *Diatrypella nigro-annulata* this specimen closely resembles that form. It has been impossible to assign any recent collections to this species, but in the Herbarium of the Royal Botanic Gardens, Kew, there are several British specimens identified as synonyms of this species. The specimen of *Sphaeria aspera* (Fr.) Petrak, in the Herbarium of M. C. Cooke, appears to be exactly similar to *Diatrypella verruciformis* on *Corylus*, while in the same herbarium the specimen of *Diatrypella aspera* on elm closely resembles the *D. tocciaeana* form. In the Herbarium of F. Currey the specimen of *Sphaeria aspera*, Bentham Hill 1856, appears to be identical with *Diatrypella nigro-annulata*, and the specimen of *Sphaeria aspera*, Weybridge, September 1855, is in its external appearance of the *Diatrypella favacea* type.

It can be seen from the above account that different stromatal forms corresponding to the species listed by Massee (1887) exist, but the differences between these forms are small and not clearly significant. *D. favacea* on *Betula* is distinct in that the stromata are usually ovoid, but on a single branch of *Betula* stromata typical of both *Diatrypella favacea* and *D. verruciformis* have been found. The white disk supposed to be characteristic of *D. favacea* may be clearly seen in some stromata but absent from other stromata in the same collection. *D. nigro-annulata* and *D. tocciaeana* differ essentially from *D. verruciformis* only in that the stromata are smaller and less erumpent through the periderm of the substratum. In certain specimens of *D. verruciformis* on *Corylus* some of the stromata are small and closely covered by the periderm so that they resemble those of *Diatrypella tocciaeana*. As pointed out by von Höhnel (1915), the black marginal line surrounding the stromata, which is the chief diagnostic character of *D. nigro-annulata*, is visible only through the very thin periderm of some twigs of *Fagus* and not through the opaque periderm of *Alnus*. The black line is present beneath the periderm of all this group of 'species' and therefore does not distinguish *Diatrypella nigro-annulata* from the other forms. The specimens examined demonstrate that there is no definite difference which can be used to distinguish the stromata of *D. aspera* from those of *D. nigro-annulata* and *D. verruciformis*.

An attempt has been made to find characters other than stromatal appearance which might be used to separate these species. In addition to the Herbarium specimens cited above, a detailed examination was made of over seventy British specimens. These specimens are in the Herbaria of W. B. Grove, E. W. Mason and C. G. C. Chesters. Twelve of them are on *Alnus*, twenty-one on *Betula*, fifteen on *Corylus*, one on *Salix* and twenty-two on *Fagus*. The number of perithecia in a single stroma varies with the size of the stroma, ranging from five to twenty on *Betula* and *Corylus*, and from

two to eight on *Alnus* and *Fagus*. The perithecia are similar in form in all specimens. They are ovoid to orbicular, tapering into long narrow necks, black-walled 500–1000 × 400–700 μ. The asci (Figs. 1A, G, 2A, G) are narrow clavate, tapering into long slender stalks, thin-walled except at the apices. In some asci an inverted cone-shaped spore canal may be seen in the thick wall at the apex. The size range of the sporing part of the ascus and of complete asci from the specimens examined is given in Table 1. The

Table 1. *Diatrypella favacea* (Fr.) Ces. & de Not.

Source	Sub-stratum	Asci (in μ) Length	Asci (in μ) Sporing part	Ascospores (in μ)
Rabenhorst, *Fung. Eur.* 253	?*Alnus*	—	—	6–8 × 1–2
Herb. Mason 255	*Alnus*	—	60–98 × 10–11	5–8 × 1–2
256	*Alnus*	—	70–103 × 9–11	6–8 × 1–2
Herb. Grove, Heythrop, Oxon. 27. xi. 32	*Alnus*	158–234	80–125 × 10–12	6–8 × 1–2
Herb. Chesters 279	*Alnus*	160–230	90–130 × 11–13	6–9 × 1–1·5
280	*Alnus*	135–216	78–110 × 10–12	6–8 × 1–2
281	*Alnus*	—	65–126 × 8–10	5–8 × 1–2
282	*Alnus*	—	80–127 × 8–10	6–8 × 1–2
283	*Alnus*	142–200	76–102 × 10–12	5–9 × 1–2
Fries, *Scler. Suec.* 306	?*Betula*	—	—	6–8 × 1–2
Fuckel, *Fung. Rhen.* 1040	*Betula*	—	—	6–8 × 1–2
Herb. Mason 302	*Betula*	125–180	60–95 × 8–10	6–8 × 1–2
303	*Betula*	115–190	68–85 × 8–10	6–8 × 1–1·5
307	*Betula*	132–235	72–108 × 8–11	6–8 × 1–2
311	*Betula*	130–200	65–105 × 8–10	6–8 × 1–2
Herb. Chesters 290	*Betula*	162–210	66–96 × 9–12	6–8 × 1–2
291	*Betula*	170–210	68–95 × 9–11	6–8 × 1–1·5
298	*Betula*	158–270	64–120 × 8–12	6–8 × 1–2
299	*Betula*	—	76–118 × 9–12	6–8 × 1–1·5
300	*Betula*	132–220	67–108 × 8–11	6–8 × 1–2
302	*Betula*	—	—	6–8 × 1–2
277	*Betula*	—	65–91 × 10–11	6–8 × 1–2
Fuckel, *Fung. Rhen.* 1038	?*Corylus*	—	—	6–8 × 1–2
Rabenhorst, *Fung. Eur.* 135	?*Corylus*	—	—	6–8 × 1–2
Klotzsch. *Herb. Myc.* 728	*Corylus*	—	—	6–8 × 1–2
Moug. & Nestl. *Stirp. Crypt.* 867	?*Corylus*	—	—	6–8 × 1–2
Herb. Mason 400	*Corylus*	140–205	60–110 × 8–10	6–8 × 1–2
401	*Corylus*	150–225	65–105 × 8–10	6–8 × 1–2
554	*Corylus*	135–215	70–110 × 8–10	6–8 × 1–2
1343	*Corylus*	130–205	65–110 × 8–11	6–8 × 1–2
Herb. Chesters 306	*Corylus*	145–220	70–104 × 9–11	6–8 × 1–2
307	*Corylus*	168–240	78–112 × 9–11	6–8 × 1–2
310	*Corylus*	132–218	68–108 × 8–11	6–8 × 1–2
311	*Corylus*	168–265	72–108 × 8–11	6–8 × 1–2
x	*Corylus*	198–266	110–200 × 9–12	6–14 × 1–2
792	*Salix*	135–210	65–108 × 9–12	6–8 × 1–2
Rabenhorst, *Fung. Eur.* 1822	*Fagus*	—	—	6–8 × 1–2
Herb. Mason 551	*Fagus*	150–200	80–120 × 8–10	6–8 × 1–2
552	*Fagus*	170–245	90–130 × 8–10	6–8 × 1–2
563	*Fagus*	155–200	80–105 × 7–10	6–8 × 1–2
564	*Fagus*	145–200	70–115 × 7–11	6–8 × 1–1·5
567	*Fagus*	130–195	76–108 × 8–11	6–8 × 1–2
Herb. Grove, Heythrop, Oxon. 11. i. 31	*Fagus*	—	70–110 × 9–12	6–8 × 1–2
Herb. Chesters 317	*Fagus*	156–200	84–122 × 9–12	6–9 × 1–1·5
318	*Fagus*	—	82–116 × 8–12	6–8 × 1–2
319	*Fagus*	148–202	72–124 × 7–10	6–8 × 1–2
321	*Fagus*	—	74–112 × 8–11	6–8 × 1–2
323	*Fagus*	—	75–110 × 8–11	6–8 × 1–2
325	*Fagus*	156–210	60–98 × 8–10	6–8 × 1–2

Twenty asci and fifty ascospores were measured from each specimen.

table demonstrates that there is considerable variation in size of both the sporing part of the asci and of the complete asci in each specimen. The differences in size range between different specimens cannot be correlated with the type of stroma or with the species of the substratum. The complete size range is of the same order on each substratum; differences between individual specimens are probably largely due to asci of maximum and minimum sizes not being measured in each specimen. There seems no justification for the statement of Nitschke (1867) that the asci of *Diatrypella favacea* are constantly smaller than those of *D. verruciformis* and *D. nigro-annulata*. Only one specimen, No. x in Herbarium C. G. C. Chesters, had asci of the same size range as that given by Nitschke for *D. verruciformis*, and that specimen was abnormal in having exceptionally large stromata and occasional large ascospores. Moreover, both Berlese (1905) and Traverso (1906) gave measurements for the sporing part of the ascus of *D. verruciformis* which are similar to those given by Nitschke for *D. favacea*. Fusiform asci, as described by Fries (1846) for *D. verruciformis*, have not been seen. It has also been impossible to confirm Nitschke's observation that the asci of *D. favacea* and *D. nigro-annulata* differ from the asci of the other species in this group in being narrower at the apex.

The ascospores in all the specimens examined are unicellular, cylindrical, rounded at the ends, only slightly curved, dilutely fuscous, usually 6–8 × 1–2 μ (see Table 1). Nitschke (1867) stated that the ascospores of *D. favacea* are generally smaller than those of *D. verruciformis* but his measurements differ only in that the ascospores of *D. favacea* are given as 1·5 μ wide and those of *D. verruciformis* as 1·5–2 μ wide. This difference was not found in the specimens examined. The same author described the spores of *D. tocciaeana* as 5–7 μ long, and regarded them as significantly shorter than those of the related species. In the specimens examined in which the stromata are typical of *D. tocciaeana* the majority of the ascospores are 6–8 μ long, and ascospores as short as 5 μ have been seen in specimens of each of the stromatal forms.

It thus appears that *D. favacea*, *D. verruciformis*, *D. nigro-annulata*, *D. tocciaeana* and *D. aspera* can be separated from each other only by differences in the habit of the stromata. These differences are small and not clearly defined. In section IV of this study further evidence is presented to show that the separation of the species on this criterion cannot be justified.

(2) *Diatrypella quercina* (Pers. ex Fr.) de Not. ex Cooke

The following specimens in the Herbarium of the Royal Botanic Gardens, Kew, have been examined:

Rabenhorst, *Fung. Eur.* Nos. 319 and 635.
Fuckel, *Fung. Rhen.* No. 1037 (sub.nom. *Microstoma enteroleucum* Awld.).
Sphaeria quercina Persoon 'Bellovaco 1821, in quercia No. 6 ill. Persoon vidit'.

In each of these specimens the perithecial stromata are similar in appearance. They are black, almost cylindrical, and distinctly erumpent through the host periderm. The surface of the stromata is rather flat except for the slightly projecting perithecial ostioles. The ostioles are three to five sulcate or with a number of less clearly marked furrows. The stromata vary

considerably in size, being from 2 to 4 mm. in diameter, and from 1 to 2 mm. high. Only incomplete polysporous asci were seen. The ascospores are unicellular, cylindrical, rounded at the ends, strongly curved, slightly fuscous, 8–12 × 2–3 μ.

Twenty-six British specimens from the herbaria of E. W. Mason and C. G. C. Chesters have been examined. Of these twenty-three are on *Quercus*, two on *Castanea* and one on *Fagus*. There is no variation in form on these three hosts, and all the stromata closely resemble those described above. The asci are narrowly clavate with long thin stalks and are thin-walled except at the apices (Fig. 3A). The sporing part of the ascus varies from 70 to 135 μ long by 8 to 14 μ wide, the width being slightly greater than that given by Nitschke (1867). There is still greater variation in the length of the complete ascus which ranges from 120 to 240 μ long (Table 2). In

Table 2. *Diatrypella quercina* (Pers. ex Fr.) de Not. ex Cke.

Source	Substratum	Asci (in μ) Length	Asci (in μ) Sporing part	Ascospores (in μ)
Fuckel, *Fung. Rhen.* 1037	*Quercus*	—	—	8–12 × 2–3
Rabenhorst, *Fung. Eur.* 319	*Quercus*	—	—	8–12 × 2–3
Fung. Eur. 635	*Quercus*	—	—	8–12 × 2–3
Herb. Cke. 'Bellovaco' 1821	*Quercus*	—	—	9–12 × 2–3
Herb. Mason 852	*Quercus*	156–222	80–114 × 10–12	9–12 × 2–3
853	*Quercus*	150–210	70–95 × 10–12	10–12 × 2–3
854	*Quercus*	150–203	82–122 × 10–12	8–12 × 2–3
855	*Quercus*	179–214	89–138 × 10–12	9–12 × 2–3
1248	*Quercus*	176–192	82–110 × 10–12	9–12 × 2–3
1344	*Quercus*	164–224	80–126 × 10–12	9–12 × 2–2·5
548	*Fagus*	158–188	64–100 × 10–12	9–12 × 2–3
Herb. Chesters 264	*Quercus*	145–200	64–110 × 10–12	9–12 × 2–2·5
265	*Quercus*	—	72–116 × 10–12	9–12 × 2–3
270	*Quercus*	—	75–160 × 9–11	8–12 × 2–3
271	*Quercus*	125–216	65–118 × 8–11	8–12 × 2–3
272	*Quercus*	150–210	80–110 × 8–11	8–11 × 2–3
275	*Quercus*	120–200	80–120 × 10–12	8–12 × 2–3
276	*Castanea*	140–190	70–110 × 9–11	8–12 × 2–3
828	*Quercus*	—	80–120 × 9–12	8–12 × 2–3
780	*Castanea*	160–200	70–95 × 8–10	7–11 × 2–3

Twenty asci and fifty ascospores were measured from each specimen.

all the specimens the ascospores (Fig. 3B) are similar in size and shape to those previously described. The size and marked curvature of the ascospores is of great importance in separating this species from *Diatrypella favacea*. The figures of *Sphaeria verruciformis* published by Currey (1857) have caused confusion as they were undoubtedly drawn from a specimen of *Diatrypella quercina*.

III. The conidia

(1) *Experimental methods*

In order to study the life histories of specimens collected in the field, cultures were obtained from single ascospores, groups of ascospores and single asci of each of the reputed species. A modification of the method described by Wehmeyer (1923) was usually employed for making the isolations. A spore suspension was prepared on a sterile slide and sprayed on to a plate of clear malt agar from a capillary pipette. The plate was

then placed at an angle to allow the suspension to flow over the surface of the agar. By making the spore suspension on a slide it was possible to examine it under the microscope and see if mature ascospores were present, and also roughly to adjust the dilution of the suspension. Subcultures were grown on various media including Leonian's medium (Leonian, 1924), 2 % malt extract, oat sucrose, and wholemeal maize agars. The 2 % malt extract agar proved to be the most satisfactory and was the one most frequently used. Both autoclaved and fresh wood were inoculated from subcultures. The first inoculations were made by placing a small piece of autoclaved wood on the surface of a culture growing vigorously on an agar slope. This method was unsatisfactory due to the density of the mycelium growing over the surface of the wood. A better method was to place the pieces of wood upright on subcultures on a layer of agar at the bottom of wide-necked conical flasks. The best results were obtained by using freshly cut, surface-sterilized pieces of wood about 12 in. long, and up to $1\frac{1}{2}$ in. in diameter. The space between the wood and the neck of the flask was plugged with sterile cotton-wool. When autoclaved wood was used the pieces were small enough to be entirely within the flask, and after mycelium was well established in the wood the twigs were transferred to sterile tubes of moist sand. If a layer of agar about $\frac{1}{2}$ in. deep was used in the flasks it was possible to keep the fresh wood for a longer period in the flasks without the culture drying out and then to place the wood out of doors.

(2) *Experimental results*

(i) *Diatrypella favacea*

Conidia of *D. favacea* were described by de Notaris (1863) and by Nitschke (1867). In both cases the conidia were long, filiform and curved. Nitschke also found conidial stromata of *D. tocciaeana* but did not describe them. A second type of conidia, 4–6 × 1 μ, was described by Fuisting (1867) for *D. favacea*. Wehmeyer (1925) has grown in culture an American form of *D. favacea* and obtained conidial fructifications which appear to be identical with those described by de Notaris and Nitschke. He did not see small conidia of the type described by Fuisting.

For the purpose of this investigation isolations were made from specimens of *D. favacea* on *Alnus*, *Betula*, *Corylus*, *Fagus* and *Salix*. The collections used included specimens with stromata typical of each of the alleged species *Diatrypella favacea*, *D. verruciformis*, *D. nigro-annulata* and *D. tocciaeana*. No differences in the appearance or the growth rate of the cultures was observed which could be correlated with the stromatal forms from which the isolations were made. The optimum temperature for growth was from 20 to 25° C. In all the isolations a flat white mycelium developed. Occasionally, especially on oat-sucrose agar, the medium was coloured dirty olive or black.

Small cushion-like aggregations of mycelium, 1–2 mm. in diameter by 1 mm. high, were formed on the surface of the agar. These sometimes remained sterile for up to twelve months and were probably similar to the structures described by Brefeld (1891). In other cases spores were formed in them two to three weeks after the pustules first appeared. If a piece of

sterile wood was placed in the culture spore tendrils sometimes appeared. The spores were of two types (Figs. 1 E, M, 2 D, O); long filamentous,

Fig. 1. A–F, *Alnus* form of *Diatrypella favacea* (*D. tocciaeana*). A, asci; B, ascospores; C, sporophore bearing macroconidia from agar culture; D, sporophore bearing microconidia from agar culture; E, macro- and microconidia from agar culture; F, microconidia from inoculated wood. G–O, *Betula* form of *Diatrypella favacea*. G, asci; H, ascospores; J, sporophores bearing macroconidia from agar culture; K, sporophore bearing macroconidia from inoculated wood; L, sporophore bearing macroconidia from herbarium specimen; M, macro- and microconidia from agar culture; N, macro- and microconidia from inoculated wood; O, macro- and microconidia from herbarium specimen. Magnification: A and G, scale *a*; C, D, J, K and L, scale *b*; B, E, F, H, M, N and O, scale *c*.

slightly curved macroconidia and short, weakly curved microconidia. Measurement from tip to tip gives little indication of the actual length of the macroconidia. In order to determine their real length the spores were

drawn at a magnification of 1000 and the length of the drawings was measured with a flexible scale which could be bent to fit the outline of the

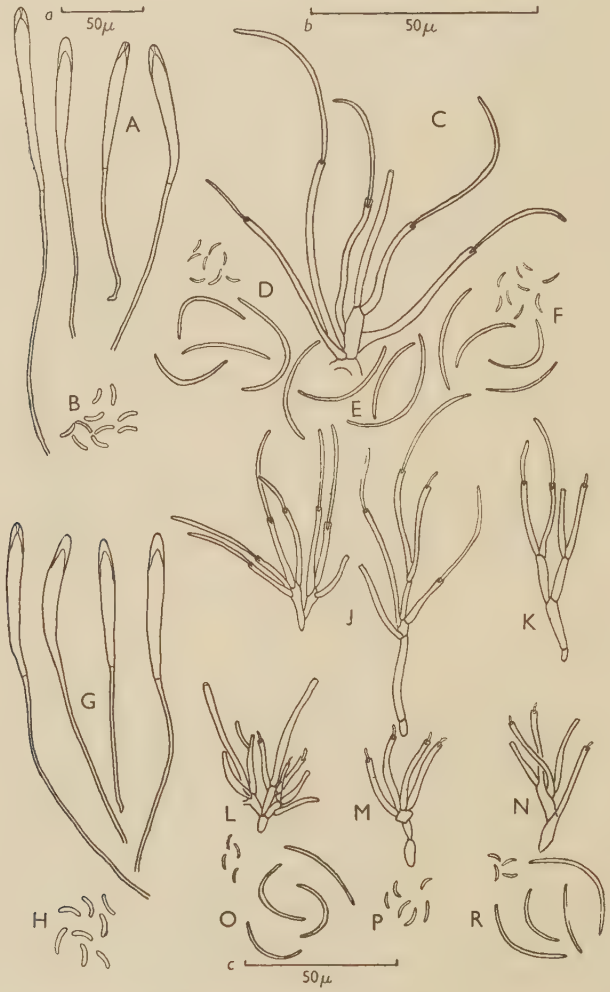


Fig. 2. A–F, *Corylus* form of *Diatrypella favacea* (*D. verruciformis*). A, asci; B, ascospores; C, sporophore bearing macroconidia from agar culture; D, macro- and microconidia from agar culture; E, macroconidia from herbarium specimen; F, macro- and microconidia from inoculated wood. G–R, *Fagus* form of *Diatrypella favacea* (*D. nigro-annulata*). G, asci; H, ascospores; J, sporophores bearing macroconidia from agar culture; K, sporophore bearing macroconidia from herbarium specimen; L, sporophore bearing microconidia from agar culture; M, sporophore bearing microconidia from inoculated wood; N, sporophore bearing microconidia from herbarium specimen; O, macro- and microconidia from agar culture; P, microconidia from inoculated wood; R, macro- and microconidia from herbarium specimen. Magnification: A and G, scale *a*; C, J, K, L, M and N, scale *b*; B, D, E, F, H, O, P and R, scale *c*.

spores. Measurements of spores obtained in this way are given in Table 3. The macroconidia varied in size from 17 to 40 by 1·5 μ, the usual range in

any one culture being 20–35 × 1·5 μ. The microconidia measured 4–10 × 1 μ, the majority being 5–8 × 1 μ. The two types of spores are quite distinct and no intermediate forms were seen. Both kinds were found in a single pustule but more often some pustules produced macroconidia and other microconidia. In some cultures only one type of spore was found at first; the other type appearing at a much later date. This indicates that, in an investigation of this kind, it is important to have a number of cultures of each fungus studied and to keep the cultures under observation for a considerable time. It was impossible to determine the factors which influenced the production of either macro- or microconidia. Every isolation seemed potentially capable of producing each type of spore but not necessarily both at the same time.

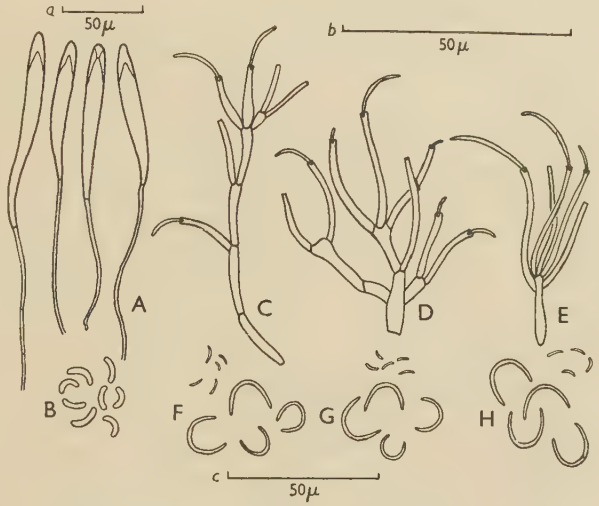


Fig. 3. *Diatrypella quercina*. A, asci; B, ascospores; C, sporophore bearing macroconidia from agar culture; D, sporophore bearing macroconidia from inoculated wood; E, sporophore bearing macroconidia from herbarium specimen; F, macro- and microconidia from agar culture; G, macro- and microconidia from inoculated wood; H, macro- and microconidia from herbarium specimen. Magnification: A, scale *a*; C, D and E, scale *b*; F, G and H, scale *c*.

The sporing pustules had an irregular cavity surrounded by a hymeniun composed of branched sporophores. These sporophores were examined by teasing out a little of the hymenium in water and staining in 1 % aqueous congo red. The sporophores bearing macroconidia (Figs. 1 C, J, 2 C, J) were longer and more frequently branched than those bearing microconidia (Figs. 1 D, 2 L) which often arose in groups from a single basal cell. Each branch terminated in a growing point, surrounded by a collar, from which the macro- or microconidia were produced. This type of meristem was described by Pethybridge & Lafferty (1917) as occurring in *Fusarium coeruleum* and the spores were of the type classified as phialospores by Mason (1933). The outer layer of the fructification consisted of closely interwoven hyphae never organized into a definite pycnidial wall. The hymenial layer was often very much folded and the cavity became pseudolocellate in pustules which produced only microconidia.

A close comparison was made of the conidial fructifications formed in cultures isolated from stromata typical of *Diatrypella tocciaeana* on *Alnus*, *Diatrypella favacea* on *Betula*, *Diatrypella verruciformis* on *Corylus* and *Salix* and *Diatrypella nigro-annulata* on *Fagus*. No differences could be detected in the form of the pustules or in the structure of the sporophores in any of the cultures. The microconidia were similar in size and shape in all the isolations. There was no apparent difference in the curvature of the macroconidia (Figs. 1 E, M, 2 D, O). In order to compare the spore sizes in different isolations spores were measured from subcultures, on 2 % malt agar, which were as nearly as possible of the same age. Spores were measured from monospore and multispore cultures isolated from six different collections on *Alnus* and *Betula* and eight different collections on *Corylus* and *Fagus*. In all, nearly one hundred cultures were used. The range of size of the spores varied in different cultures, but the variation was as great between spores from cultures isolated from the same stromatal forms as it was between spores from cultures isolated from different stromatal forms. A summary of the conidial measurements is given in Table 3. From Graph 1 it can be seen that the curves for distribution of length of macroconidia from isolations from *Alnus*, *Betula*, *Corylus* and *Fagus* correspond closely.

Pieces of sterile and fresh wood were inoculated with cultures from each of the four forms of *Diatrypella favacea* but macroconidia were seen only on three pieces of wood. Two of these were pieces of *Corylus* inoculated with cultures from *Diatrypella verruciformis* type of stromata; the third was a piece of *Betula* inoculated with a culture from a specimen typical of the form of *D. favacea* usually found on that wood. Sterile wood was inoculated and pustules appeared after keeping the wood in tubes for three months. Small, almost flat or slightly raised, conical pustules were formed beneath the substratum periderm, often on the sides of young perithecial stromata (Fig. 4C). Each pustule consisted of a labyrinthiform cavity lined by a layer of sporophores. At first the sporophore layer almost completely surrounded the cavity, but later the upper part of the layer was broken so that the cavity was covered by the periderm. In some cases the conidial apparatus persisted until the perithecial stromata were mature, taking the form of a number of furrows arranged along the axes of an ellipse, on the sides of the stromata beneath the substratum periderm. Below the sporophores was a network of closely interwoven hyphae which did not form a definite wall but gradually became less compact, the mycelium eventually intermingling with the host tissues. The sporophores (Fig. 1 K) were similar in structure to those produced on agar and bore filamentous macroconidia (Figs. 1 N, 2 F), 23–50 × 1 μ. On one piece of *Corylus* and on the *Betula* twig pustules of a second type were present. They were similar in form to the others but were pseudolocellate (Fig. 4 D) and contained slightly curved microconidia 4–10 × 1 μ, borne on short sporophores. In a few pustules both macro- and microconidia were present. The macro- and microconidia were similar in shape on both hosts and had a similar range in size (see Table 3). The frequency distribution curves for the macroconidia from the *Betula* and *Corylus* isolations are shown in graph 2 and coincide fairly closely.

In another experiment cultures from the 'species' *Diatrypella favacea*,

Table 3. *Measurements of conidia of* Diatrypella favacea

Collection	Source of conidia	Substratum	Macroconidia (in μ)	Microconidia (in μ)
	1. *Alnus* form (= *D. tocciaeana*)			
Herb. Chesters 279	Multispore culture	*Alnus*	—	4–8 × 1
279	Multispore culture	*Betula*	—	5–8 × 1
279	Multispore culture	*Corylus*	—	4–9 × 1
279	Multispore culture	*Fagus*	—	4–8 × 1
279	Multispore culture	Malt agar	15–29 × 1–2	4–9 × 1
280	2 ascospore cultures	Malt agar	18–42 × 1–2	—
281	9 ascospore cultures	Malt agar	19–42 × 1–2	4–10 × 1
281	1 ascospore culture	Oat-sucrose agar	19–37 × 1–2	—
282	2 ascospore cultures	Malt agar	20–40 × 1–2	—
283	1 ascospore culture	Malt agar	19–37 × 1–2	—
Inoculated *Alnus*	2 ascospore cultures	Malt agar	16–30 × 1–2	—
	2. *Betula* form (= *D. favacea*)			
299	Herbarium specimen	*Betula*	23–49 × 1–2	4–9 × 1
302	Ascospore culture	*Betula*	23–50 × 1–2	4–9 × 1
302	Ascospore culture	*Betula*	—	4–10 × 1
302	Ascospore culture	*Alnus*	—	4–9 × 1
302	Ascospore culture	*Corylus*	—	4–8 × 1
302	Ascospore culture	*Fagus*	—	4–8 × 1
299	Ascus culture	*Betula*	—	4–8 × 1
290	2 ascospore cultures	Malt agar	17–39 × 1–2	—
291	3 ascospore cultures	Malt agar	18–37 × 1–2	—
291	1 ascospore culture	Oat-sucrose agar	22–39 × 1–2	—
298	1 ascospore culture	Malt agar	19–42 × 1–2	—
299	2 ascospore cultures	Malt agar	19–42 × 1–2	4–9 × 1
300	2 ascospore cultures	Malt agar	21–35 × 1–2	4–8 × 1
302	1 ascospore culture	Malt agar	20–40 × 1–2	—
277	3 ascospore cultures	Malt agar	17–38 × 1–2	—
277	1 ascospore culture	Oat-sucrose agar	18–33 × 1–2	—
	3. *Corylus* form (= *D. verruciformis*)			
311	Herbarium specimen	*Corylus*	23–50 × 1–2	—
306	Herbarium specimen	*Corylus*	—	4–9 × 1
307	Herbarium specimen	*Corylus*	—	4–8 × 1
310	Ascospore culture	*Corylus*	23–50 × 1–2	4–8 × 1
311	Ascospore culture	*Alnus*	—	4–7 × 1
311	Ascospore culture	*Betula*	—	4–8 × 1
311	Ascospore culture	*Fagus*	—	4–9 × 1
306	2 ascospore cultures	Malt agar	18–39 × 1–2	—
307	6 ascospore cultures	Malt agar	17–47 × 1–2	—
310	2 ascospore cultures	Malt agar	18–37 × 1–2	—
311	3 ascospore cultures	Malt agar	21–38 × 1–2	4–8 × 1
311	2 ascospore cultures	Oat-sucrose agar	18–42 × 1–2	—
×	6 ascospore cultures	Malt agar	17–47 × 1–2	—
Inoculated *Corylus*	2 ascospore cultures	Malt agar	21–43 × 1–2	—
	4. *Fagus* form (= *D. nigro-annulata*)			
325	Herbarium specimen	*Fagus*	24–47 × 1–2	—
325	Herbarium specimen	*Fagus*	32–51 × 1–2	4–9 × 1–2
320	Herbarium specimen	*Fagus*	25–51 × 1–2	—
317	Multispore culture	*Fagus*	—	4–11 × 1
317	Multispore culture	*Alnus*	—	4–10 × 1
317	Multispore culture	*Betula*	—	4–10 × 1
317	Multispore culture	*Corylus*	—	4–10 × 1
317	Multispore culture	*Quercus*	—	4–9 × 1
317	Multispore culture	Malt agar	34–47 × 1–2	4–11 × 1
318	3 ascospore cultures	Malt agar	20–38 × 1–2	—
321	4 ascospore cultures	Malt agar	22–42 × 1–2	—
325	3 ascospore cultures	Malt agar	21–37 × 1–2	—
Inoculated *Fagus*	2 ascospore cultures	Malt agar	20–32 × 1–2	—

Note. Fifty microconidia were measured in each case. Fifty macroconidia were measured from each specimen on wood and twenty from each agar culture.

D. verruciformis, *D. nigro-annulata*, and *D. tocciaeana* were cross inoculated on to twigs of *Alnus*, *Betula*, *Corylus*, *Fagus* and *Quercus*. The microconidia developed readily on the four first-named hosts and once on *Quercus*. No macroconidia were seen, although the twigs were kept under observation

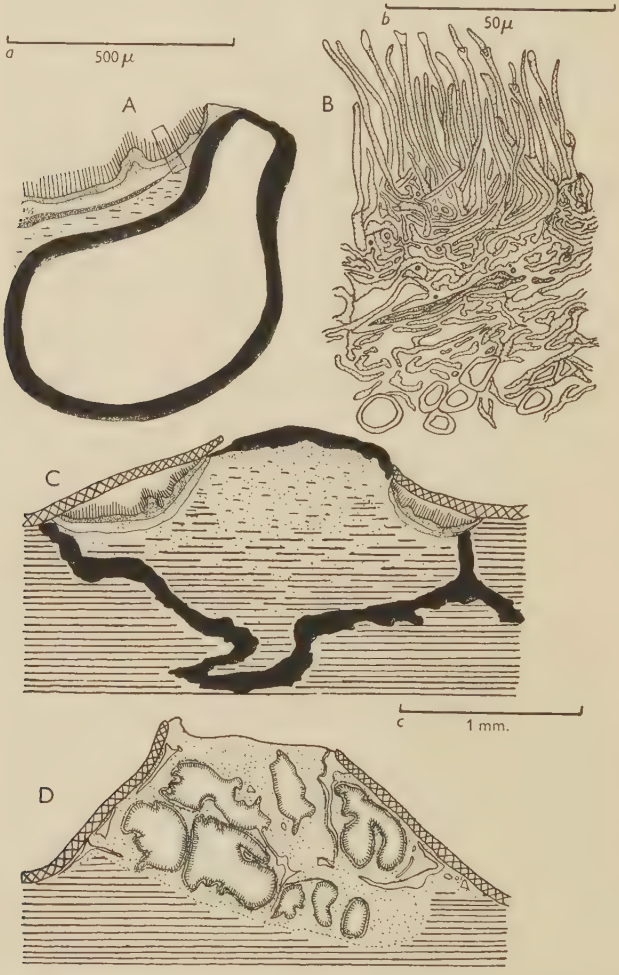


Fig. 4 Sections of conidial pustules of *Diatrypella quercina* and *D. favacea*. A, vertical section showing conidial pustule on side of old perithecium of *D. quercina*; B, drawing showing details of part of A enclosed in rectangle; C, longitudinal section showing conidial pustules on sides of young perithecial stroma of *D. favacea*; D, longitudinal section of microconidial pustule of *D. favacea*. Magnification: A, scale *a*; B, scale *b*; C and D, scale *c*.

for over a year. The pustules and sporophores resembled those previously seen and did not differ in any of the substrata. The spores issued from the pustules in slimy, orange or red masses, and were hyaline, slightly curved, 4–11 × 1 μ, the majority measuring 5–9 × 1 μ (Table 3).

In spite of a careful search macroconidia associated with perithecial

stromata were seen in only four collections now in Prof. Chester's Herbarium. On *Betula* (No. 299), on *Corylus* (No. 311), and on *Fagus* (Nos. 320, 325), the macroconidia were found on the sides of old perithecial stromata as described by Tulasne (1863). On removal of the periderm the conidial pustules were seen as a group of sulcae arranged in an ellipse (Fig. 5B).

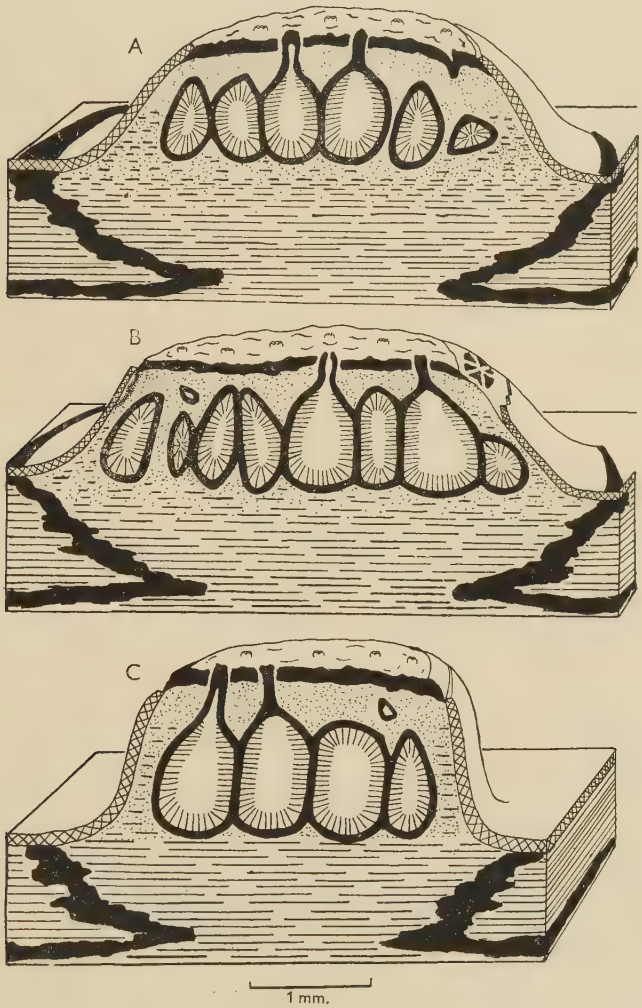
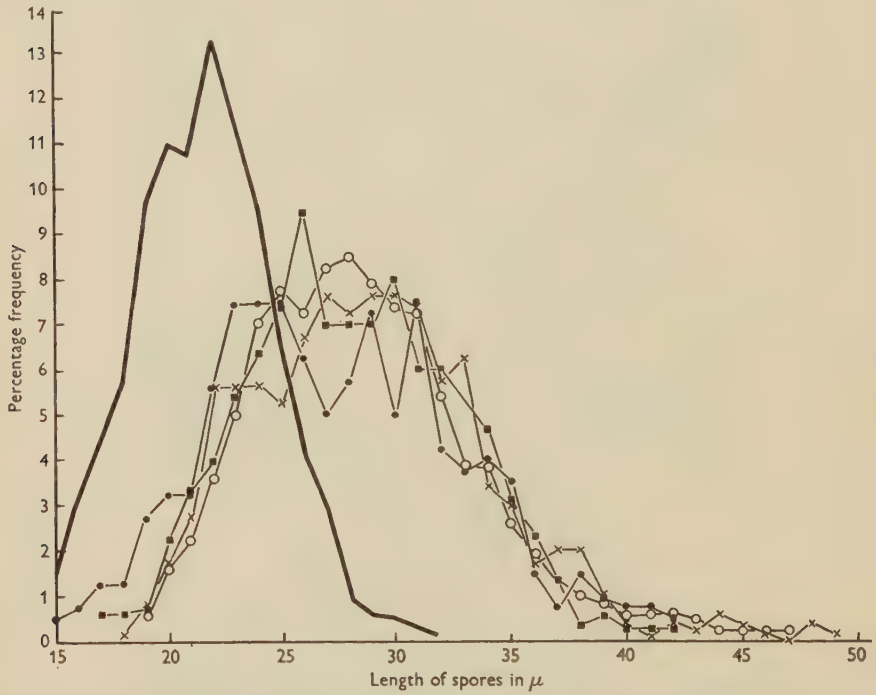


Fig. 5. Diagrams of stromata of *Diatrypella favacea* and *D. quercina*. A, *D. favacea*; B, *D. favacea* showing conidial pustules on the sides of a perithecial stroma; C, *D. quercina*.

No definite pycnidial wall was present, though in some cases the outer hyphal layer of the pustule was darker than the remainder. The sporophores (Figs. 1L, 2K, N) were of the usual type. The macroconidia were similar in size and curvature to those produced on inoculated wood (Figs. 1O, 2E, R). There was no significant difference in the size of the

conidia in each of the three specimens (Table 3). Microconidia (Figs. 1 O, 2 R) were present in the same pustules as the macroconidia in the specimens on *Betula* and *Fagus* and were in two specimens on *Corylus*. They were of the same shape and size, 4–10 × 1 μ, on all three substrata. Up to the present no conidia have been found associated with perithecial stromata of the *Diatrypella tocciaeana* form.

The similarity of the conidial material produced in isolations made from *D. favacea*, *D. verruciformis*, *D. nigro-annulata* and *D. tocciaeana* supports the view that these species are not separable. The few collections of conidia associated with perithecial stromata in the field provide further evidence for uniting these species.

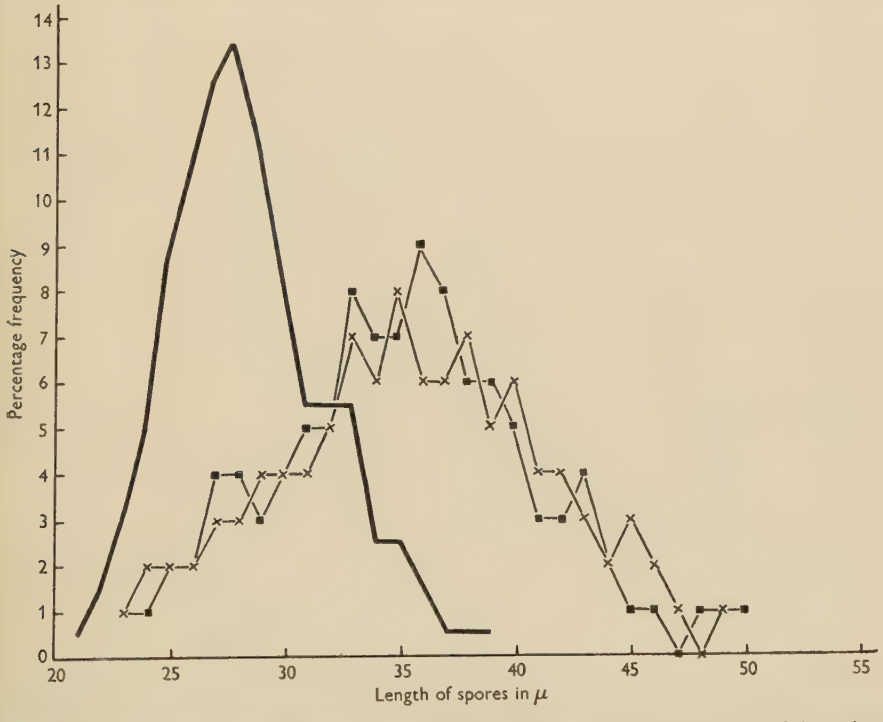


Graph 1. Percentage frequency-spore length curves for macroconidia from agar cultures of *Diatrypella favacea* and *D. quercina*. KEY: ●——●——●, *D. favacea*, *Alnus* form (= *D. tocciaena*); ■——■——■, *D. favacea*, *Betula* form (= *D. favacea*); ×——×——×, *D. favacea*, *Corylus* form (= *D. verruciformis*); ○——○——○, *D. favacea*, *Fagus* form (= *D. nigro-annulata*); ————————, *D. quercina*.

The microconidia described above probably correspond with the conidia seen by Fuisting (1867). The macroconidia appear to be the same as those obtained in culture by Wehmeyer (1925). They are also probably identical with the conidia described by Tulasne (1863) and Nitschke (1867), and to which Traverso (1906) applied the name *Libertella favacea* Trav. As the macro- and microconidia are part of the one fungus, *Diatrypella favacea*, it is undesirable to apply separate names to them especially as, unless perithecia are present, identification of the macroconidia is extremely difficult and of the microconidia impossible.

(ii) *Diatrypella quercina*

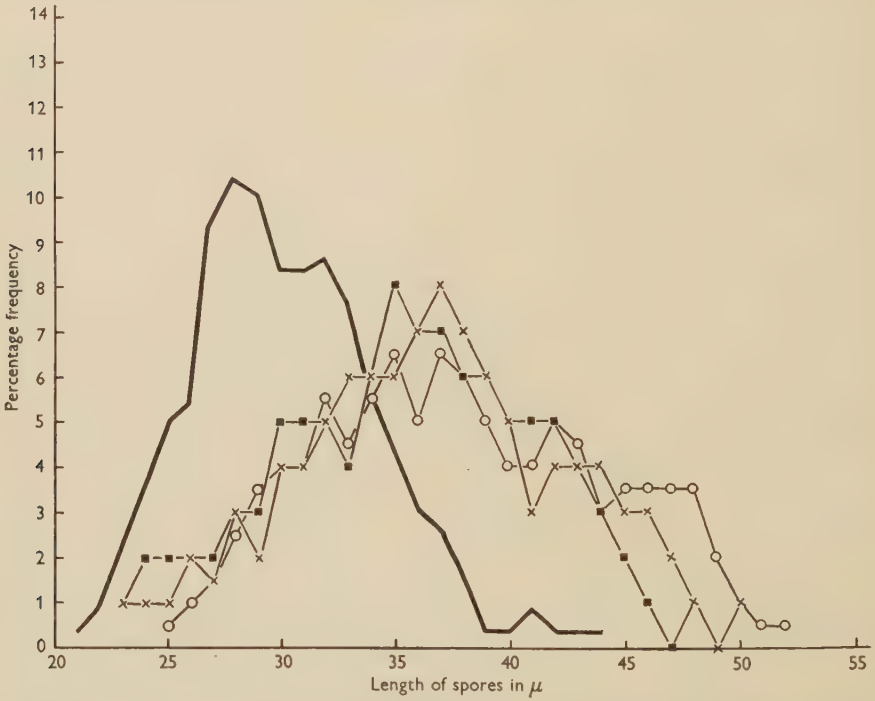
Tulasne (1863) and Nitschke (1867) have both described conidia associated with perithecial stromata of *D. quercina*. These conidia were long, very slender, filiform, cylindrical, curved. Nitschke (1867) also observed a second type of conidial fructification, although he did not include a description of it in his Latin diagnosis. On stromata of *D. quercina* he found a golden brown, powdery covering consisting of conidia 8–12 × 1½ μ arising on sterigmata 28 μ long. These conidia were similar but rather smaller than ascospores of *D. quercina* and the majority were very slightly curved. Fuisting (1867) expected to find polymorphism in the conidia of *D. quercina* but was able to see only a few cylindrical spores of a dubious nature among



Graph 2. Percentage frequency-spore length curves for macroconidia from wood inoculated with *Diatrypella favacea* and *D. quercina*. KEY: ■——■——■, *D. favacea*, *Betula* form (= *D. favacea*); ×——×——×, *D. favacea*, *Corylus* form (= *D. verruciformis*); ————, *D. quercina*.

the common long conidia. *D. quercina* was grown in culture by Brefeld (1891) but only sterile mycelial cushions developed. Wehmeyer (1925), in America, has grown in culture a fungus which he cites as *D. quercina* (Pers.) Nits. f. *crataegi* E. & E. and has obtained a conidial stage which he has placed in the genus *Cytosporina*. Dr Wehmeyer has kindly sent me specimens of this fungus from his herbarium, and I am of the opinion that it is not identical with the species described in this study. The stromata are smaller and less erumpent, and the ascospores less strongly curved.

Isolations of *Diatrypella quercina* were made from several collections on *Quercus* and one on *Castanea*. The ascospores germinated in 24–48 hr. and the culture developed as a fine white mycelium over the surface of the agar. The optimum temperature for growth was from 25 to 30° C. Small cushion-like aggregations of mycelium, similar to those formed in cultures of *Diatrypella favacea*, appeared in one to three months. These structures became rather pink and from them extruded yellow to orange spore tendrils. These were composed mainly of long, filiform, very strongly curved conidia (Fig. 3F). The curvature of the spores was entirely different from that of the corresponding macroconidia of *D. favacea*. The macro-conidia were measured by the same method as was used for the macro-



Graph 3. Percentage frequency-spore length curves for macroconidia from herbarium specimens of *Diatrypella favacea* and *D. quercina*. KEY: ■——■——■, *D. favacea*, *Betula* form (=*D. favacea*); ×——×——×, *D. favacea*, *Corylus* form (=*D. verruciformis*); ○—○—○, *D. favacea*, *Fagus* form (=*D. nigro-annulata*); ————————, *D. quercina*.

conidia of *D. favacea*, and the dimensions are given in Table 4. The frequency-length curve for these conidia is entirely distinct from the corresponding curves for the macroconidia of *D. favacea* (Graph 1). The majority of the conidia were 20–25 × 1 μ, but in a number of tendrils microconidia 4–8 × 1 μ were also present. Such microconidia were not seen in all subcultures, and in others they were very few, but in one or two cultures they were more numerous than the macroconidia. As in *D. favacea* no intermediate forms were seen. The pustules were similar in structure to

those described for *D. favacea*; the sporophores (Fig. 3C) were also of the same type as those occurring in that species.

Conidial fructifications developed readily on both sterile and non-sterile wood inoculated with monospore and multispore cultures. The pustules (Fig. 4A, B) were similar in structure to those of *D. favacea* and again had no true pycnidial wall. The sporophores (Fig. 3D) were similar to those produced on agar. Both macroconidia and microconidia were formed and, unlike those of *D. favacea*, they exuded from the pustules in slimy spore tendrils. These spores (Fig. 3G) were similar to those produced in agar cultures, but the macroconidia on the wood substratum were constantly slightly longer, measuring 20–40 μ, the majority being 24–34 μ long (see Table 4).

Table 4. *Measurements of conidia of* Diatrypella quercina *(Pers. ex Fr.) de Not. ex Cke.*

Collection	Source of conidia	Substratum	Macro-conidia (in μ)	Micro-conidia (in μ)
Herb. Mason 1248	Herbarium specimen	*Quercus*	24–36 × 1–1·5	—
1344	Herbarium specimen	*Quercus*	22–34 × 1–1·5	—
Herb. Chesters 270	Herbarium specimen	*Quercus*	23–41 × 1–2	—
272	Herbarium specimen	*Quercus*	24–41 × 1–2	4–8 × 1
275	Herbarium specimen	*Quercus*	24–43 × 1–2	—
828	Herbarium specimen	*Quercus*	28–38 × 1–2	—
265	Multispore culture	*Quercus*	23–36 × 1–2	—
270	Multispore culture	*Quercus*	21–40 × 1–2	—
271	Monospore culture	*Quercus*	23–40 × 1–2	5–9 × 1
276	Monospore culture	*Quercus*	22–35 × 1–2	—
264	8 ascospore cultures	Malt agar	15–29 × 1–2	—
264	1 ascospore culture	Malt agar	15–27 × 1–2	4–8 × 1
265	2 ascospore cultures	Malt agar	15–26 × 1–2	5–9 × 1
265	1 ascospore culture	Oat-sucrose agar	17–30 × 1–2	5–7 × 1
271	6 ascospore cultures	Malt agar	15–31 × 1–2	5–7 × 1
271	8 ascospore cultures	Oat-sucrose agar	18–29 × 1–2	—
276	4 ascospore cultures	Malt agar	15–28 × 1–2	—
828	4 ascospore cultures	Malt agar	15–33 × 1–2	—

Note. Fifty microconidia were measured in each case except for specimen 272 in which only twenty were measured. Fifty macroconidia were measured in each case from *Quercus*. On agar twenty macroconidia were measured from each culture.

Conidia associated with perithecial stromata were seen in six collections (Table 4). In two, both macro- and microconidia were found in the same spore tendrils. They may have been present in the other specimens, but overlooked, as it is difficult to see the microconidia when they are mingled with a large number of macroconidia. The form of the pustules, the structure of the sporophores (Fig. 3E) and the size and shape of the conidia (Fig. 3H) were similar to that described on inoculated wood. There was some variation in the size range of the macroconidia from different collections, but this is probably due to the fact that the fifty spores measured did not cover the whole size range of the large number of spores present in a single pustule.

The macroconidia appear to be identical with those described by Tulasne (1863) and Nitschke (1867). To this conidial stage Traverso (1906) gave the name *Cytosporina quercina* (Nits.) Trav. while Grove (1937) stated that the conidial stage described by Tulasne was *Libertella quercina* Grove. The

structure of the conidial pustules is characteristic of the genera *Libertella* Desm. and *Naemospora* Pers. which differ from each other only in that the spores in *Libertella* are long and those in *Naemospora* short. However, as in *Diatrypella favacea*, there appears to be no reason for applying separate names to the conidial stages.

In order to obtain further information about these species, attempts were made to develop cultures from the conidia. Macro- and microconidia were kept in sterile distilled water, tap water, in extracts made from wood and on the surface of pieces of wood of *Alnus*, *Betula*, *Corylus*, *Fagus* and *Quercus* for periods up to three months but germination never took place. Attempts to induce germination by keeping the spores at low temperatures and at alternating high and low temperatures also failed, so that the part played by these spores in the life histories of the species remains unknown.

IV. The perithecia in culture

No perithecial stromata were formed on any of the agar media used, but mature perithecia were obtained on sterile and fresh host wood inoculated with monospore and multispore cultures of *Diatrypella quercina* and *D. favacea*. The inoculations were carried out by the methods described in the previous section. A detailed investigation of the development of the perithecia has not been made and this section is devoted more to the variation in appearance of the perithecial stromata on different woods and under different external conditions.

(1) *Diatrypella favacea*

Perithecial stromata were formed on sterile wood, inoculated in ordinary culture tubes and then transferred to sterile tubes containing moist sand, but were covered by a felt-like layer of mycelium. When the perithecia were mature the mycelium was penetrated by smooth, black stromata. Stromata of this type developed on pieces of *Alnus*, *Betula*, *Corylus* and *Fagus* wood inoculated with each of the forms of *Diatrypella favacea*, but mature asci were seldom produced.

More satisfactory results were obtained by inoculating larger pieces of wood in flask cultures and then placing them out of doors, as in the open the superficial mycelium disappeared. Pieces of wood of *Alnus*, *Betula*, *Corylus*, *Fagus* and *Quercus* were inoculated with cultures isolated from stromata typical of *Diatrypella favacea*, *D. verruciformis*, *D. nigro-annulata* and *D. tocciaeana*. In all cases the stromata were irregular, frequently confluent, and only slightly erumpent through the wood periderm. The surface of the stromata was black and rather smooth, except for the small, slightly projecting, sulcate ostioles. The stromata were not typical of any form of *D. favacea* seen in the field but most closely resembled some of the specimens collected on *Alnus*. Mature ascospores were formed in eight to twelve months after inoculation of the wood. In some cases the perithecial stromata were preceded by the microconidia but more often no conidia were seen.

In another series of cross-inoculations on sterile wood a different method was used. A block of agar containing a single germinating ascospore was

inserted beneath a flap in the periderm of the wood. The flap was then replaced over the agar and covered by a piece of sterile moist cotton-wool. The wood was then replaced into a sterile tube containing moist sand. By using this method little superficial mycelium developed and mature stromata formed in three to four months without taking the wood out of the tubes. It was found, however, that this method was only successful when the wood was inoculated during the months of March to August inclusive. Apparently during the winter months food or fungal growth-promoting substances essential for the growth and reproduction of this species were not available in the wood. The stromata formed were similar in appearance in all cases. They were small, obtusely conical, sometimes confluent, often scarcely erumpent through the wood periderm, but with the prominent black ostioles always free. They most closely resembled the stromata usually found on *Alnus* and *Fagus*.

In order to compare the form of the stromata on different species of woods under more natural conditions a series of cross-inoculations was made on fresh wood. Freshly cut twigs about 12 in. long were inoculated in flask cultures and placed out of doors in a spinney as soon as the mycelium was well established in the wood. Pieces of the same branches which had not been inoculated were placed with them as controls. None of these controls were invaded by *Diatrypella* species so that it may be assumed that the stromata formed on the inoculated wood developed from the cultures with which they were inoculated and not by chance infection from outside sources. The variation in appearance of the stromata produced in these experiments was so great that it is necessary to discuss them in some detail.

Monospore and multispore cultures of the *D. tocciaeana* form produced stromata similar in form on *Alnus*, *Betula*, *Corylus* and *Fagus*. These stromata were minute, often confluent, and scarcely erumpent through the periderm. They were much smaller than the stromata of *Diatrypella* usually found in Britain and were often of the type described for the genus *Cryptovalsa* (Ces. & de Not.) Fuckel. On one twig of *Fagus* inoculated with a multispore culture the stromata were typical of *Diatrypella nigro-annulata*, but a monospore culture isolated from the same perithecium and inoculated on to the same substratum species produced stromata of the very small kind.

Isolations from the fungus on *Betula* gave more varied results. One multispore culture, isolated from a specimen typical of *Diatrypella favacea*, as it was maintained by Nitschke, gave rise to stromata on *Alnus*, *Corylus* and *Fagus* which were respectively typical of *Diatrypella tocciaeana*, *D. verruciformis* and *D. nigro-annulata*. On *Betula* the same culture produced two kinds of stromata. Those on the upper side of the wood had the white disk and black six sulcate ostioles characteristic of *D. favacea*, but on the lower side in contact with the ground the stromata were entirely black and might have been assigned to *D. verruciformis*. Stromata were also formed on *Quercus* and these most closely resembled those of *D. tocciaeana*. A monospore culture isolated from the same specimen produced on *Alnus* and *Fagus* stromata of the type usually found on those hosts in the field but on *Betula* and *Corylus* the stromata resembled those formed on most of the wood inoculated with *Diatrypella tocciaeana* cultures. A similar series of cross-inoculations was made using cultures isolated from black stromata on

Betula. On *Alnus*, *Betula*, *Corylus* and *Fagus* the stromata were typical of those usually found on these species in the field.

Cultures isolated from stromata of the *Diatrypella verruciformis* type on *Corylus*, and the *Diatrypella nigro-annulata* form on *Fagus* were inoculated on to the same range of species and gave similar results. The stromata formed on each substratum species either closely resembled those usually found on that species or were of the small, scarcely erumpent, confluent form previously described.

Cultures of any of the forms of *Diatrypella favacea* seldom produced stromata when inoculated on to *Quercus* wood. When they were formed they were small and only slightly erumpent, and in no way resembled the cylindrical, distinctly erumpent stromata of *D. quercina*.

In all inoculations in which mature stromata were formed the asci were similar in size and shape to those found in herbarium specimens. The ascospores were invariably only slightly curved and had the size range 6–8 × 1–2 μ, characteristic of *D. favacea*. Cultures started from these ascospores produced on agar the imperfect stages of *D. favacea* already described.

It is not possible to give a complete explanation for the formation of all the different forms of stromata in *D. favacea*, but there is some indication that it is influenced by the rate of growth of the fungus through the wood. If the mycelium penetrated through the wood rapidly the small, almost completely immersed type of stromata were produced in almost every case. The stromata appeared almost simultaneously along the whole length of the inoculated twig and were usually mature in six to eight months from the date of inoculation. On the other hand, when the stromata were typical of those usually found on the species of wood inoculated, development was slow. The mycelium seldom penetrated through the whole length of the wood, and stromata were formed at the end of the branch which had been in contact with the agar culture. Seven to twelve months elapsed before mature ascospores were found in the perithecia. The time taken for the formation of mature stromata varied somewhat with the time of year, development being most rapid on wood inoculated during spring and early summer. This lends support to the theory that some of the substances in the wood which are necessary to the fungus may be absent or present in insufficient amounts during the winter months. Another factor influencing the appearance of the stromata may be the external environment. In the moist atmosphere of a tube the stromata which developed resembled most closely those usually collected on *Alnus* which grows in wetter situations than any of the other species on which *Diatrypella favacea* is found. Variations in external conditions, particularly humidity, may account for the variations in form on a single substratum species. Stromata formed on the end of a branch of *Betula* close to where it was attached to the tree had the typical white disk but at the other end of the branch, which was lying in a damp ditch, the stromata were completely black. Similarly, on inoculated wood the stromata nearest the ground were black while those exposed to the air had the white disk. Thus it is suggested that the differences in external form of the stromata of *D. favacea* in different collections are due not only to differences in the physical and chemical constitution of the

substratum species but also to variations in the external environment and the rate of development of the fungus.

(2) *Diatrypella quercina*

Inoculation of small twigs by placing them in cultures on agar slopes was unsuccessful, as only masses of mycelium developed on the wood. More satisfactory results were obtained by inoculating sterile pieces of wood at least $\frac{1}{2}$ in. in diameter in flask cultures. Using this method perithecial stromata burst through the wood periderm in two to three months after placing the wood in the flasks. The stromata were cylindrical or angular, smooth on top and dirty brown. They were frequently, but not invariably, associated with the conidia. Perithecial initials were present in the stromata but no further development took place until the wood was placed out of doors. The stromata then turned black and sulcate ostioles appeared. Asci were formed in from six to ten months, and mature ascospores in eighteen months to two years from the date of inoculation. Similar results were obtained by inoculating freshly cut, surface-sterilized wood.

Monospore and multispore cultures of *D. quercina* were inoculated on to pieces of wood of *Alnus*, *Betula*, *Corylus*, *Fagus* and *Quercus*. Mature perithecia were formed on sterile and fresh wood of all species. In all cases the stromata were distinctly erumpent through the periderm and typical of small stromata of *Diatrypella quercina*. The asci had the same range of size as those in herbarium specimens and the ascospores, always strongly curved, measured 9–12 × 1–2 μ.

V. Discussion

(1) *The conidial stage*

The conidia of the two species described are of two types—long, filamentous, curved, hyaline macroconidia and short only slightly curved, hyaline microconidia. These spores are meristem phialospores as defined by Mason (1933), and extrude from the sporing pustules in gelatinous, yellow to orange or red spore masses. The sporophores from which macroconidia are produced differ from those giving rise to microconidia only in greater length and in degree of branching. The sporophores form a hymenial layer which is not bounded by a definite pycnidial wall. These pustules are open, labyrinthiform cavities characteristic of the two form genera *Libertella* Desm. and *Naemospora* Pers. (Fig. 4 C, D). These two genera were separated by Desmazières (1830), who retained the name *Naemospora* for forms with small conidia and erected the new genus *Libertella* to include the forms with long and more or less curved conidia. As both types of conidia have been shown to occur in the life history of a single species and may be formed in the same pustule, this separation into two genera seems to be unjustifiable. It appears probable that a number of the species included in these two genera may be micro- and macroconidial stages of a single fungus.

The macroconidia of *Diatrypella quercina* are usually shorter and are always more strongly curved than those of *D. favacea*. No differences could be detected in the conidia produced in cultures isolated from stromata typical of each of the 'species' *D. favacea*, *D. verruciformis*, *D. nigro-annulata*

and *D. tocciaeana*. Nor was it possible to distinguish between the conidia associated with perithecial stromata of *D. favacea*, *D. verruciformis* and *D. nigro-annulata* in the field. This evidence indicates that *D. quercina* is a distinct species but that the different forms united here under the name *D. favacea* are not separate species.

(2) *The perithecial stage*

Five of the species in Massee's list, *D. verruciformis*, *D. favacea*, *D. nigro-annulata*, *D. tocciaeana* and *D. aspera*, cannot be distinguished from each other. Their separation depends only on differences in the appearance of the stromata. Examination of many specimens has shown that the variation in stromatal form of these 'species' is so great that it cannot be used to differentiate them. Cross-inoculation experiments, in which cultures isolated from stromata typical of *D. favacea*, *D. verruciformis*, *D. nigro-annulata* and *D. tocciaeana* were grown on various species of wood, have confirmed this view. Under certain conditions a single isolation from one 'species' has produced perithecial stromata typical of the other 'species'. *D. aspera* was not included in the experiments as it was not possible to distinguish this species from the *D. nigro-annulata* form in the specimens seen. There thus seems no reason why these five forms should not be united into a single species. From Nitschke's description (1867) it seems likely that *D. meleana* is merely another form of this species but without seeing an authentic specimen no definite statement can be made.

Owing to the confusion in the synonomy some justification is necessary for the adoption of the name *D. favacea*. In the literature the only species with a similar conception to that presented here for *D. favacea* appears to be *Diatrype verruciformis* as described by the brothers Tulasne (1863). However, when de Notaris (1863) founded the genus *Diatrypella* he included *D. favacea* but not *D. verruciformis*. He also included *D. tocciaeana*, but as *D. favacea* was the first-mentioned species this name has been retained. Traverso (1906) stated that *D. favacea* de Not. on *Corylus* was really *D. verruciformis*. This confusion may have arisen owing to the fact that the '*D. verruciformis*' form usually occurs on *Corylus* while typical '*D. favacea*' stromata are found on *Betula*. The specimen which I have seen of *Sclerom. suec.* No. 306, which is one of the exsiccati cited by de Notaris, certainly appears to be on *Betula*. As it has been shown in this study that *Diatrypella favacea* and *D. verruciformis* are synonyms, and as *D. favacea* was the name transferred to *Diatrypella* by de Notaris (1863) it should take priority over all other names. In both the subgenus and the genus *Diatrypella*, *D. favacea* is the first described species and should be regarded as the type species.

Of the remaining species of *Diatrypella* listed by Massee (1887), *D. quercina* is clearly separated from the others by the strongly curved ascospores, 8–12 × 2–3 μ. The cylindrical or slightly angular stromata which are distinctly erumpent through the substratum periderm also distinguish it from the other species. This characteristic form of the stromata is not dependent upon the species of wood on which it develops as typical stromata were formed when cultures of *D. quercina* were inoculated on to pieces of wood other than *Quercus*.

The description and illustrations of *D. rousselii*, given by de Notaris (1863), indicate that this fungus is identical with *D. quercina*. The name *D. rousselii* has not been adopted by later authors and as *Sphaeria quercina* Pers. was accepted by Fries (1823) the specific epithet *quercina* should be retained. *Sphaeria quercina* was first transferred to *Diatrypella* by Cooke (1866). The specimens on which this investigation is based agree closely with a specimen in the Cooke Herbarium which Persoon had seen. There can thus be little doubt that the fungus described in this study can be cited as *D. quercina* (Pers. ex Fr.) Cke.

The genus *Diatrypella* was placed by von Höhnel (1918) in the Diatrypeen of the Allantosphaeriaceen. Wehmeyer (1926) united *Diatrypella* with *Cryptovalsa*, another genus of the Diatrypeen. It has not been possible to obtain living specimens of *Cryptovalsa* and so investigate this genus in culture, but some evidence has been obtained to support Wehmeyer's view. The effuse nature of the stroma is the only character separating *Cryptovalsa* from *Diatrypella* and, in some experiments, cultures of *D. favacea* inoculated on to wood have produced effuse stromata. In a few collections on *Alnus* I have also seen stromata of this type. Owing to the kindness of Dr Wehmeyer and Dr Miller in sending me specimens I have examined American species of *Diatrypella* in which the stromata are effuse so that there does appear to be a gradation in this genus from forms like *D. quercina* in which the stromata are almost invariably isolated to species in which the stromata are effuse. Therefore in this study I have adopted Wehmeyer's emended diagnosis for the genus *Diatrypella* except that the references to ectostroma and entostroma have been omitted. I regard this separation of the stroma into two parts as artificial, and I have not been able to see two distinct zones in the stromata which I have examined. I have also appended to Wehmeyer's diagnosis a description of the conidial pustules found in the genus.

VI. Summary

Diatrypella de Not. in Cesati & de Notaris emend. Wehmeyer (1926).

1. Stroma effuse or isolated. Stroma often pustulate but usually not widely erumpent, bounded by a dark marginal zone. Perithecia usually clustered, rarely separately erumpent. Ostioles usually sulcate. Asci long stalked, polysporous. Spores allantoid, one celled, yellow hyaline.

2. Imperfect stromata open labyrinthiform grooves, lined by a hymenium of closely crowded, branched sporophores which produce slimy, meristem phialospores. Microconidia hyaline, short, slightly curved. Macroconidia long, filamentous, curved, hyaline.

Type species: *D. favacea* (Fr.) Cesati & de Notaris.

(*a*) Ascospores slightly curved 6–8 × 1–2 μ, *D. favacea*.
(*b*) Ascospores strongly curved 8–12 × 2–3 μ, *D. quercina*.

For the synonomy of these two species I am greatly indebted to Mr E. W. Mason and Miss J. Hickman.

(*a*) *Diatrypella favacea* (Fr.) Ces. & de Not. emend.

1. *Sphaeria favacea* Fr. in *K. svenska VetenskAkad. Handl.* **37**, 150, 1816.
   Sphaeria favacea Fr. ex *Syst. myc.* **2**, pt. 2, p. 354, 1823.
   Diatrype favacea (Fr.) Fr. *Summa Veg. Scand.* pt. 2, p. 385, 1849.
   Diatrype favacea (Fr.) Fr. in Ces. & de Not. *Comment. Soc. Crittogam. Ital.* **1**, pt. IV, p. 202, 1863, in sect. II *Diatrypella*.
   Diatrypella favacea (Fr.) Ces. & de Not. in de Not. *Sfer. Ital.* p. 29, 1863.

   Type collection:
   Locus natalis. In truncis *Betulae*.
   Locus classicus. Sweden.
   Lecto-type: Fries, *Scler. suec.* No. 306.

2. *Diatrypella tocciaeana* de Not. *Sfer. Ital.* p. 30, 1863.
   Type collection:
   Locus natalis. In ramis *Alni incanae*.
   Locus classicus. Italy.

3. *Sphaeria aspera* Fr. *Syst. myc.* **2**, pt. 2, p. 354, 1823.
   Diatrype aspera (Fr.) Fr. *Summ. Veg. Scand.* pt. 2, p. 385, 1849.
   Diatrypella aspera (Fr.) Nits. *Pyr. Germ.* **1**, 74, 1867.

   Polytypic:
   (*a*) Locus natalis. In caudice *Fagi*, Schumacher *Enum. Pl. Saell.* **2**, 170, 1803 sub. *S. verrucaeformis* Pers.
   Locus classicus. Denmark.
   (*b*) Locus natalis. Ad cortices *Fagi*, aliarumque arborum: Fries.
   Locus classicus. Sweden.

4. (i) *Sphaeria verrucaeformis* Ehrhart ex Pers. *Synopsis Fung.* p. 26, 1801.
   (ii) *Sphaeria verrucaeformis* Ehrhart ex Fries, *Syst. myc.* **2**, pt. 2, p. 355, 1823.
   (iii) *Diatrype verrucaeformis* (Ehrh.) Fr. *Summ. Veg. Scand.* pt. 2, p. 385, 1849.
   (iv) *Diatrypella verrucaeformis* (Ehrh.) Nits. *Pyr. Germ.* **1**, 76, 1867.
   (v) *Sphaeria avellanae* Pers. in Roemer, *Neues Magazin für die Botanik*, **1**, 82, 1794=*S. verrucaeformis* Ehrh. ex Pers., fide Persoon 1801.

   (i) Polytypic:
   (*a*) Locus natalis. Bark (probably *Betula*): Ehrhart, Plant cryptog. No. 280 (coll. between 1792 and 1799). Locus classicus. Herrenhausen, nr. Hanover, Germany.
   (*b*) Locus natalis. In ramis *Coryli avellanae*: Persoon type of *Sphaeria avellanae* Pers.

   (iii) and (iv) Monotypic. Ehrhart, Plant. cryptog. No. 280.

5. *Stromatosphaeria nigro-annulata* Grev. *Fl. Edin.* p. 358, 1824.
   Diatrypella nigro-annulata (Grev.) Nits. *Pyr. Germ.* p. 81, 1867.

Type collection:
Locus natalis. Dead branches of *Tilia*.
Locus classicus. Scotland.

(A) Perithecial stromata varied in habit, ovoid orbicular or irregular angular, sometimes confluent, more or less erumpent through periderm

with ostioles free, dirty brown or black, sometimes with a white disk. Perithecia three to twenty in a single stroma, closely crowded, monostichous or occasionally distichous, ovoid orbicular, tapering into long, narrow necks with three to six sulcate ostioles, 300–1000 μ in diameter. Asci narrow clavate, long stalked, thin-walled except at apices, polysporous, 60–130 × 8–11 μ sporing part, 130–250 μ total length. Spores unicellular, cylindrical, rounded at ends, slightly curved, dilutely fuscous, 6–8 × 1–2 μ.

(B) Conidial stromata, flat, sulcate, without definite wall. Conidiophores branched producing slimy, meristem phialospores. Microconidia short, hyaline, slightly curved, 4–9 × 1 μ. Macroconidia long, filamentous, somewhat curved, hyaline, 25–50 (majority 35–45) × 1 μ. On *Alnus*, *Betula*, *Corylus*, *Fagus* and *Salix*.

(*b*) *Diatrypella quercina* (Pers. ex Fr.) de Not. ex Cke.

1. *Sphaeria quercina* Pers. in Roehmer's *Neues Magazine für die Botanik*, p. 82, 1794.

Sphaeria quercina Pers. ex Fr. *Syst. myc.* **2**, pt. 2, p. 362, 1823.
Stromatosphaeria quercina (Pers.) Grev. *Fl. Edin.* p. 358, 1824.
Diatrype quercina (Pers.) Fr. *Summ. Veg. Scand.* pt. 2, p. 385, 1849.
Diatrypella quercina (Pers. ex Fr.) de Not. ex Cooke in *J. Bot.* **4**, 99, 1866.

Type collection.
Locus natalis. In ramis *Quercus*.
Locus classicus. (?) Holland.

2. *Diatrype rousselii* Ces. & de Not. in *Comment. Soc. Crittogam. Ital.* **1**, pt. IV, p. 201, 1863.

Diatrypella rousselii (Ces. & de Not.) de Not. *Sfer. Ital.* p. 31, 1863.

Type collection:
Plantus exsicc. sub *Sphaeria quercina* Pers. in Herb. Roussel.
2 = 1: Fide Nits. *Pyr. Germ.* **1**, 71, 1867.
Fide Sacc. *Syll. Fung.* **1**, 206, 1882.
Fide Trav. *Fl. Ital. Crypt.* p. 1, p. 71, 1906.

(A) Perithecial stromata cylindrical or angular, erumpent through closely adhering periderm, surface at first rather flat, roughened later, brownish becoming black. Perithecia three to fifteen in a single stroma, ovoid or spherical, tapering into long necks with four sulcate ostioles. Asci narrow clavate, tapering into long, narrow stalks, many spored 65–100 × 8–12 μ sporing part, 130–220 μ total length. Spores unicellular, strongly curved, cylindrical, rounded at ends, dilutely fuscous, 9–12 × 2–3 μ.

(B) Conidial stromata sulcate, open cavities, without distinct wall, frequently associated with young perithecial stromata or persisting on sides of older perithecial stromata. Conidiophores branched, producing serial phialospores. Microconidia short, hyaline, slightly curved 4–8 × 1 μ. Macroconidia long, filamentous, very strongly curved, hyaline, 20–45 (mostly 25–35) × 1–2 μ.

On *Quercus*, *Castanea* and *Fagus*.

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ON THE NOMENCLATURE AND IDENTITY OF THE COLIFORM SOFT-ROT BACTERIA

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The coliform soft-rot bacteria have been given many names by various investigators ever since L. R. Jones (1901) called the bacterium responsible for the soft-rot of carrots, *Bacillus carotovorus*. In succeeding years the following names were proposed for the potato blackleg pathogen: *B. phytophthorus* (Appel, 1902), *B. atrosepticus* (van Hall, 1902), *B. solanisaprus* (Harrison, 1907) and *B. melanogenes* (Pethybridge & Murphy, 1911). The bacteria causing similar rots of other host plants, likewise received different specific names. Thus, Harrison (1902) proposed the name *B. oleraceae* for the bacterium producing soft-rot in cauliflower, van Hall (1903) *B. omnivorus* for the pathogen causing soft-rot of iris rhizomes, and Townsend (1904) *B. aroideae*, for the bacterium responsible for soft-rot of calla lily. These investigators considered that the bacteria they described and named were distinct species and not identical with any previously recorded.

Harding and Morse (1909) studied forty-three strains of coliform soft-rot bacteria, including type cultures of *B. carotovorus*, *B. phytophthorus*, *B. aroideae*, *B. omnivorus* and *B. oleraceae*. They found some variability in the carbohydrate fermenting powers of the various strains, particularly in the formation of gas in the fermentation of sugars. They did not consider, however, that the differences warranted the separation of the species, which they regarded as variants of *B. carotovorus*.

Morse (1917) concluded from his studies of potato blackleg that *B. atrosepticus*, *B. solanisaprus* and *B. melanogenes* were identical.

As the generic name, *Bacillus*, can no longer be used for non-sporing rod-shaped bacteria (*Report Second International Congress for Microbiology*, London 1936 (published 1937), and *J. Bact.* XXXIII, 1937, p. 445), the name *Bacterium*, as defined by Dowson (1939), is employed in the remainder of this paper.

Since the publications of Harding and Morse (1909) and of Morse (1917) various investigators have attempted to re-establish three species. Massey (1924) separated *Bacterium carotovorum* and *B. aroideae* on the basis of acid production by the former on 5% alcohol agar. Lacey (1926) differentiated *B. carotovorum* from *B. phytophthorum* on the basis of the fermentation of maltose by the latter only, and this was later confirmed by Dowson (1939). Lacey also distinguished the two species on serological grounds.

Leach (1930), who reviewed the literature, including his own work (Leach, 1927), concluded that *B. carotovorum* and *B. phytophthorum* were not distinct species. He noted a variability in carbohydrate fermenting powers and maintained that blackening of the infected tissue was not peculiar to strains of the *B. phytophthorum* type, as Lacey (1926) had suggested. He considered that *B. aroideae* might still be kept as a separate species on the strength of certain cultural characters and the inability to produce gas in

the fermentation of sugars. Bonde (1939) working with sixty-two strains, confirmed Leach's observations except that he recorded acid production in a glycerine medium by a number of his isolates.

Dowson (1941), in an investigation of soft-rot of vegetables, again separated the three species on the basis of differences in fermentation reactions and in pathogenicity. He stated that *B. carotovorum* did not ferment maltose; *B. phytophthorum* fermented maltose with the production of acid and gas; and *B. aroideae* fermented sugars without the production of gas. He considered that *B. carotovorum* and *B. aroideae* had a comparatively wide host range, whereas *B. phytophthorum* was restricted to the potato plant.

Elrod (1941) in a serological and biochemical investigation of eighteen strains found some degree of serological separation of maltose fermenting and maltose non-fermenting strains. This confirmed the serological work of Lacey (1926). Elrod also found variability in gas production during fermentation reactions, and in particular noted the loss of ability to form gas* in a strain kept in culture for a long period. He was able to separate the gas-producing and non-gas-producing strains because the last-named did not ferment sorbitol, and concluded that there were two 'groups' of soft-rot bacteria, presumably corresponding to *B. carotovorum* and *B. phytophthorum*. He regarded *B. aroideae* as synonymous with *B. carotovorum*, being a strain which had lost its gas-producing power. Waldee (1945), in studies on a wide range of peritrichous phytopathogenic bacteria, stated that none of the soft-rot bacteria fermented maltose. He distinguished *B. carotovorum* from *B. phytophthorum* on the grounds that the first species produced small amounts of acid in glycerol media, whereas the other produced no discernible acid. He also separated *B. aroideae* from the other two species because it did not form gas in the fermentation of carbohydrates.

I have examined the pathogenicity and biochemical characters of twenty-eight strains of coliform soft-rot bacteria in an attempt to clarify the nomenclature and identity of these bacteria.

Sources of strains

The twenty-eight strains were isolated by me except where stated, and were:

T 1J, isolated from a soft-rotted tomato plant, August 1945.

P 1J–P 16J, 292, 1152, 14262, 15262, 2463, 3063, 3263, isolated from storage rots of potato tubers between October 1945 and April 1947.

BP 1J, isolated from a blackleg potato plant, June 1947.

31D, isolated from a storage rot of celery by W. J. Dowson, 1939. (*B. carotovorum*).

138D, isolated from a blackleg potato plant by W. J. Dowson, 1939 (*B. phytophthorum*).

66D, isolated from soft-rot of tobacco stems in Uganda, by C. G. Hansford, 1936. Culture in the collection of W. J. Dowson (*B. aroideae*).

* 'Ability to form gas' is here taken to mean sufficient gas production to saturate the medium and to appear as a bubble in the Durham fermentation tube.

Pathogenicity tests

In all previous work on the pathology of soft-rot bacteria, the conditions under which tests of pathogenicity were carried out, were not standardized. I employed conditions most favourable for the spread of the parasite (high temperature and high humidity) and thereby insured that mildly pathogenic strains gave a positive test.

Plants of tomato, potato and *Arum maculatum*, potato tubers, iris rhizomes, and onions were used. Tomato seedlings were grown in pots and were inoculated when they were about six inches high. Similarly, potato shoots were allowed to grow about four inches long before inoculation. Young plants of *Arum maculatum* were collected in the field and potted. All the plants were inoculated at the bases of young petioles. Three petioles were inoculated for each test, which was repeated once or twice. The inoculum consisted of a dense suspension of bacteria prepared by allowing growth from a forty-eight hour agar-slope culture to diffuse out for twenty minutes in a small quantity of sterile water. Several loopfuls of the bacterial suspension were pricked into each petiole with a sterilized needle and the controls were inoculated with sterile water. After inoculation the plants were kept in a saturated atmosphere under bell jars, at 20° C., for forty-eight hours.

Whole potato tubers were inoculated by stabbing them with a needle carrying bacterial growth from a forty-eight hour agar-slope culture. After inoculation the tubers were kept over water in sealed chambers at 25° C. The young tips of iris rhizomes and small onions were cut into slices and inoculated in the same way. They were kept in Petri dishes with a small amount of sterile water (Dowson, 1941). The controls were stabbed with a sterilized needle.

Results

The results are shown in Table 1. All the strains tested produced a rot in iris rhizomes and onion slices and most of them produced a rot in tomato and potato plants, as well as potato tubers, irrespective of their ability to ferment maltose.

The amount of stem blackening produced in potato plants varied considerably, not only between strains, but also with the same strain in different tests. The same was true of inoculated tubers.

Only three strains out of fourteen tested caused a rot of arum petioles. These were 66 D, a strain which fermented sugars including maltose without the production of gas; P 4J, a strain which did not ferment maltose on isolation; and P 9J, a strain which had lost the power to ferment maltose after a year in culture. Both P 4J and P 9J produced gas from other sugars when first isolated.

From these tests it is clear that *B. carotovorum* and *B. phytophthorum* cannot be considered distinct species on the present basis of separation, and that *B. aroideae* cannot be satisfactorily distinguished on the basis of pathogenicity for arum petioles (Dowson, 1941), because two strains, which did not correspond to *B. aroideae* in their biochemical characters, produced a rot in this host.

Table 1. *The reactions of strains of coliform soft-rot bacteria on various hosts*

For explanation see Table 2.

Host	Potato plant	Potato tuber	Tomato plant	*Arum maculatum*	Iris rhizome	Onion bulb	Host	Potato plant	Potato tuber	Tomato plant	*Arum maculatum*	Iris rhizome	Onion bulb
T 1J	+	+	+	O	−	−	292	+	+	+	O	+	+
P 1J	+	+	+	O	−	−	14262	+	+	+	−	+	+
P 2J	+	+	+	O	+	−	15262	+	+	+	−	+	+
P 3J	O	+	+	−	+	−	1152	+	+	+	−	+	+
P 4J	+	+	+	+	+	−	2463	+	+	+	−	+	+
P 5J	+	+	+	−	+	−	3063	+	+	+	−	+	+
P 6J	+	+	+	−	+	−	3263	+	+	+	O	+	+
P 7J	+	+	+	−	+	−	BP1J	+	+	+	−	+	+
P 8J	+	+	+	−	−	−	31D	+	+	+	O	+	+
P 9J	+	+	O	+	+	−	138D	+	+	+	O	+	+
P 10J	+	+	+	O	−	−	66D	+	+	+	+	+	+
P 11J	+	+	+	−	+	−							
P 12J	+	+	+	−	−	−							
P 13J	+	+	+	O	+	−							
P 14J	+	+	+	−	+	−							
P 15J	+	+	+	−	+	−							
P 16J	+	+	+	−	+	−							

Biochemical tests

The reactions of the twenty-eight strains to certain alcohols and carbohydrates have been examined. The final acidity, as determined by the methyl-red test, and the production 2–3-butyleneglycol and acetylmethylcarbinol (acetoin) in glucose phosphate media were also investigated.

Media and methods

Alcohols

(i) *Ethyl alcohol.* Tubes of 5% ethyl alcohol agar, to which 1% bromocresol-purple had been added, were made up according to the method described by Massey (1924).

(ii) *Sorbitol and glycerol.* 1% sorbitol or glycerol was added to a basal medium (S.A.B., 1944) consisting of 0·1% ammonium dihydrogen phosphate ($NH_4H_2PO_4$), 0·02% potassium chloride (KCl), and 0·02% magnesium sulphate ($MgSO_4$), with 3% bromo-thymol-blue as an indicator. The solutions were adjusted to pH 7·0 and tubes were made up, each containing about 6 ml. of solution and a Durham fermentation tube. The tubes were sterilized by bringing momentarily to 120° C. in an autoclave, turning off the gas and allowing to cool (Davis & Rogers, 1938).

Carbohydrates

(i) *Sugars and glucosides.* Tubes of 1 % solutions of glucose, sucrose, maltose and salicin were made up in the same way as for glycerol and sorbitol.

All the above media were inoculated with a 3 mm. loop from twenty-four-hour old cultures in Difco 'bacto-tryptone'. Three tubes were used for

each test and were incubated for ten days (three weeks for tubes of ethyl alcohol agar) at 25° C. when they were examined for the formation of acid and gas.

(ii) *Starch.* The ability to hydrolyse starch was tested by growing the strains on slopes of horse-heart infusion agar to which had been added 0·2 % soluble starch. After seven days growth at 25° C. the slopes were flooded with dilute iodine solution. If the test was positive no blue-black colour formed on the surface of the slope beneath the bacterial growth.

Methyl red and Voges-Proskauer tests

Glucose phosphate medium (Min. Health, 1939) and O'Meara's (1931) modification of it were used in the investigation of the final acidity reached, and for the formation of acetylmethylcarbinol, in glucose fermentation. O'Meara stated that the addition of sodium fumarate as a hydrogen acceptor prevented the acetylmethylcarbinol from being reduced to 2–3-butyleneglycol, thus giving a negative test. Stahly and Werkman (1942) showed that the acetylmethylcarbinol, 2–3-butyleneglycol reaction was a reversible oxidation-reduction system, and they found that a high redox potential favoured the formation of acetylmethylcarbinol, and a low potential the formation of 2–3-butylene-glycol. This supports O'Meara's observation, for the addition of fumarate will produce a high redox potential in the system.

Wide tubes were used for these media to give the maximum surface for oxidation. They were autoclaved and inoculated in the manner described for sugars. After incubation for five days at 25° C. the formation of acetylmethylcarbinol was tested for by adding a small amount of creatinine (O'Meara, 1931) and 3–4 ml. of 40 % caustic soda solution to each tube.

Results

The results of the biochemical tests are shown in Table 2. All the strains grew on ethyl alcohol agar but only two (P 1 J and P 10 J) produced acid. These results, while confirming those of Leach (1930), indicate that the distinction between *B. carotovorum* and *B. aroideae*, which Massey (1924) suggested was provided by growth on this medium, is not a critical one.

All the strains produced acid and gas in sorbitol except 31 D and 66 D (Dowson's *B. carotovorum* and *B. aroideae* strains), which produced acid only. This suggests that Elrod's (1941) separation of gas-producing and non-gas-producing strains, on the basis of sorbitol fermentation, is not justified. All the strains produced acid in glycerol and this is contrary to the findings of Waldee (1945) who asserted that *B. carotovorum* produced acid in glycerol while *B. phytophthorum* did not.

All the strains isolated from potato tubers formed acid and gas in glucose, sucrose and salicin on isolation, but some of them lost the ability to form gas after a year in culture. Sixteen out of twenty-five strains isolated by me fermented maltose with the formation of acid and gas on isolation, two produced acid only and seven did not ferment maltose. After a year in culture only eight of the sixteen strains formed acid and gas and, invariably, the quantity formed was much less than in the original test. With three of

Table 2. *The reactions of strains of coliform soft-rot bacteria on various media*

g=surface growth on ethyl alcohol agar; A=acid production in fermentation reactions; G=gas Production in fermentation reactions; O signifies that the medium was not attacked in the fermentation reactions, and indicates a negative result in the M.R. and V.P. tests; −signifies that the reaction was not tested; + signifies a positive result. The figures I and II after sucrose and maltose indicate the reactions on isolation (I), and after a year in culture (II).

Host	Ethyl alcohol	Sorbitol	Glycerol	Glucose	Sucrose I	Sucrose II	Maltose I	Maltose II	Salicin	Starch	Glucose phosphate M.R.	Glucose phosphate V.P.	Fumarate M.R.	Fumarate V.P.
T 1J	g	AG	A	AG	AG	AG	AG	A	AG	O	+	+	O	+
P 1J	gA	AG	A	AG	AG	AG	AG	AG	AG	O	O	+	O	+
P 2J	g	AG	A	AG	AG	AG	AG	A	AG	O	+	O	O	+
P 3J	g	AG	A	AG	AG	AG	AG	AG	AG	O	+	O	O	+
P 4J	g	AG	A	AG	AG	A	O	O	AG	O	O	+	O	+
P 5J	g	AG	A	AG	AG	AG	AG	AG	AG	O	+	O	O	+
P 6J	g	AG	A	AG	AG	AG	AG	O	AG	O	+	+	O	+
P 7J	g	AG	A	AG	AG	AG	AG	O	AG	O	+	+	O	+
P 8J	g	AG	A	AG	AG	AG	AG	AG	AG	O	+	+	O	+
P 9J	g	AG	A	AG	AG	A	AG	O	AG	O	+	+	O	+
P 10J	gA	AG	A	AG	AG	AG	AG	AG	AG	O	+	+	O	+
P 11J	g	AG	A	AG	AG	AG	O	O	AG	O	O	+	O	+
P 12J	g	AG	A	AG	AG	AG	AG	O	AG	O	+	+	O	+
P 13J	g	AG	A	AG	AG	A	O	O	AG	O	O	+	O	+
P 14J	g	AG	A	AG	AG	AG	AG	O	AG	O	+	+	O	+
P 15J	g	AG	A	AG	AG	AG	O	O	AG	O	O	+	O	+
P 16J	g	AG	A	AG	AG	AG	AG	AG	AG	O	+	+	O	+
292	g	AG	A	AG	AG	AG	AG	AG	AG	O	−	−	O	+
14262	g	AG	A	AG	AG	AG	A	A	AG	O	−	−	O	+
15262	g	AG	A	AG	AG	A	AG	A	AG	O	−	−	O	+
1152	g	AG	A	AG	AG	AG	A	A	AG	O	−	−	O	+
2463	g	AG	A	AG	AG	AG	O	O	AG	O	−	−	O	+
3063	g	AG	A	AG	AG	AG	O	O	AG	+	−	−	O	+
3263	g	AG	A	AG	AG	A	O	O	AG	+	−	−	O	+
BP 1J	g	AG	A	AG	AG	AG	AG	AG	AG	O	−	−	O	+
31D	g	A	A	AG	AG	AG	O	O	AG	O	+	O	O	+
138D	g	AG	A	AG	AG	AG	AG	AG	AG	O	+	+	O	+
66D	g	A	A	A	A	A	A	A	A	O	O	+	O	+

these eight strains at least one tube showed no gas production in each test. Three strains produced acid only and the remaining five of the sixteen strains had lost the power to ferment maltose altogether.

These tests showed that ability to ferment maltose is a very variable character in coliform soft-rot bacteria and is, therefore, not a good criterion for distinguishing between *B. carotovorum* and *B. phytophthorum*.

66D, Dowson's *B. aroideae*, failed to form gas on all carbohydrate media tested.

Three strains, P 4J, P 9J and 66D, were subjected to passage through potato plants and tubers, and arum petioles, and were then tested for ability to ferment glucose and maltose. The biochemical tests with all three strains remained the same.

Two strains only had a slight ability to hydrolyse starch. These results agree with those of L. R. Jones (1909), who found that there was some conversion of starch to amylodextrin but no complete diastatic action.

Leach (1927) and Bonde (1939) recorded no diastatic activity in the strains that they examined but many authors, including Lacey (1926), Dowson (1939) and Waldee (1945), reported definite hydrolysis with some strains.

All the strains gave methyl red negative and V.P. positive reactions when tested in O'Meara's fumarate medium. The results in the plain glucose phosphate medium were very variable and it would appear from the number of methyl red positive tests that the coliform soft-rot bacteria do not readily form acetylmethylcarbinol in this medium. The addition of sodium fumarate apparently promotes the formation of acetylmethylcarbinol and it seems likely that, in addition to preventing the reduction of acetylmethylcarbinol, it also prevents the reduction of acetaldehyde and favours its condensation to acetylmethylcarbinol, as suggested by Kluyver, Donker and Visser't Hooft (1925).

The discrepancy in the behaviour of various strains in the two media may explain the V.P. negative test which Dowson (1939) recorded for *B. carotovorum*, and a similar result reported by Waldee (1945) for many strains.

Discussion

The pathogenicity and biochemical tests have shown that the characters at present used to separate *B. carotovorum* and *B. phytophthorum* are not satisfactory. Lacey (1926) and Elrod (1941) successfully identified two groups serologically but the evidence now provided indicates that these two groups are not correlated with specific pathological or biochemical activity. I therefore suggest that *B. carotovorum* and *B. phytophthorum* are strains of a single species, which should be called *Bacterium carotovorum* (Jones) Lehmann & Neumann. Strains isolated from blackleg plants and from soft-rotted vegetables may be expected to show slight differences, but these are not sufficient to establish separate species.

None of the strains I isolated corresponded biochemically to *B. aroideae*, or came to resemble it after a year in culture. The strain of *B. aroideae* (66D) included has never produced gas in the fermentation of sugars. However, as it remained in culture for a month after isolation before the reactions in carbohydrates were tested it is possible that during this time the ability to form gas was lost. Throughout its culture 66D has remained an extremely virulent strain. I agree with Harding and Morse, Leach, and Bonde, that gas production in the fermentation of sugars is not a good character for the separation of species. *B. aroideae* can therefore also be considered as a non-aerogenic strain of *B. carotovorum*. Dowson (1949), in an examination of the above evidence and in the light of his own experience of the group, agrees with this view.

Summary

Pathogenicity and biochemical tests were carried out with twenty-eight strains of coliform soft-rot bacteria, mainly isolated from rotting potato tubers, in an attempt to clarify the nomenclature of the group. The separation of the two species *Bacterium carotovorum* and *B. phytophthorum* on differences in pathogenicity and fermentation reactions is shown to be unsatisfactory.

B. carotovorum, *B. phytophthorum* and *B. aroideae* are considered to be strains of a single species, which should be called *Bacterium carotovorum* (Jones) Lehmann & Neumann.

This account forms part of an investigation of the bacterial rots of stored potatoes made possible by a grant from the Agricultural Research Council, to whom I make grateful acknowledgement. The work was carried out at Cambridge under the supervision of Dr W. J. Dowson for whose advice and helpful criticism I wish to express my indebtedness.

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FORECASTING OUTBREAKS OF POTATO BLIGHT IN WEST SCOTLAND

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(With 6 Text-figures)

The control of Potato Blight by spraying the foliage with fungicides demands, for complete success, some method of foreseeing the imminence of an attack by *Phytophthora infestans*. Efficacy of such 'protective' sprays is greatly enhanced if they are applied immediately before the fungus appears. Beaumont (1947) formulated a simple temperature-humidity rule to forecast the first appearance of Blight. The rule assumes that when the minimum temperature is over 50° F., and the relative humidity over 75 % for a continuous period of forty-eight hours or more, an outbreak of the disease can be expected to follow within a short period of fifteen to twenty-two days.

Results from the disease phenology plots at this Department have been used to confirm Beaumont's general premises, to make them more accurate for the west of Scotland, and to discuss practical implications of the use which might be made of such forecasts within the area.

The disease phenology plots

A range of crops grown in randomized, replicated blocks was established at Auchincruive in 1944, and since 1945 the varieties, plot size and other treatments have been standardized. Five varieties of potatoes are grown—Gladstone, Dunbar Rover, Kerr's Pink, Arran Pilot and King Edward. Seed of Gladstone and Dunbar Rover, coming originally from Scotland and Northern Ireland, was also compared, but as no significant difference in the behaviour of these variants has been found, they are included as replicates of each of the two varieties in the present investigation. There are thus seven plots (rows) in each block, and each block is replicated three times, giving twenty-one plots in all. Each row is 38 yards long each year. The amount of virus (Rugose Mosaic) began to increase in 1947. The seed stocks were 'indexed' during the following winter, and this brought the amount of Rugose Mosaic down to less than 4 % in the succeeding year.

Blight is estimated on the foliage by means of a method almost identical with the key prepared by the Society's Disease Measurement Sub-Committee. Blight on the tubers after lifting is measured by weighing the total produce, weighing all tubers with Blight, and calculating the latter as a percentage of the former. Continuous measurements of temperature and humidity are made on recording instruments in louvre screens at a height of 4 ft. The Department also maintains a Class 3 weather station and the instrumental records are useful in checking the readings of the recording instruments.

Validity of the forecasts

A combination of the records for Blight and weather allows a useful test of Beaumont's temperature-humidity rule. Typical results are shown by Figs. 1 and 2. It will be seen that in 1945 (Fig. 1) the first valid forecast was on 28–30 July; this was indeed followed sixteen days later by the first appearance of Blight on the foliage. The years 1946 and 1948 were similar.

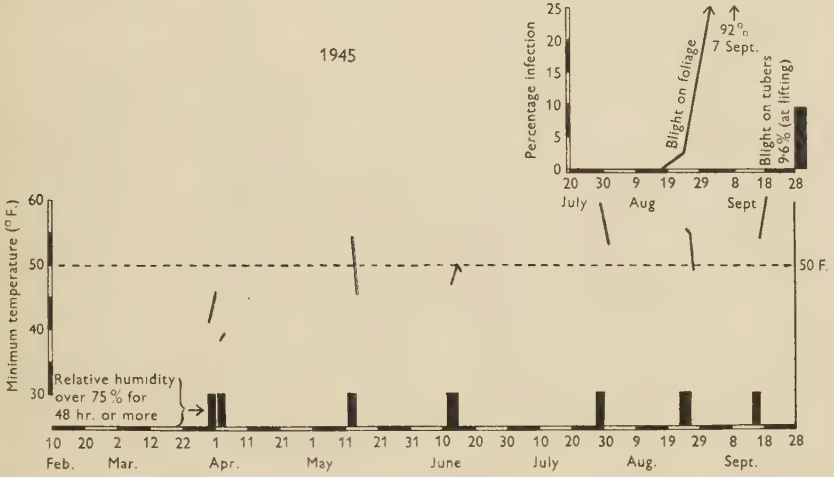


Fig. 1. The horizontal axis represents a time scale covering the active growing period of the potato. Above this are shown; (*a*) in block form, any period of forty-eight hours or more when the relative humidity was over 75%; and (*b*) fragments of the curve showing daily minimum temperature. If these occur above the dotted line (50° F.), the period should be followed by Blight in about fifteen to twenty-two days (Beaumont, 1947). The first suitable period in 1945 was 28–30 July, and this was followed by Blight on 15 August (see inset graph, to same horizontal scale). Auchincruive.

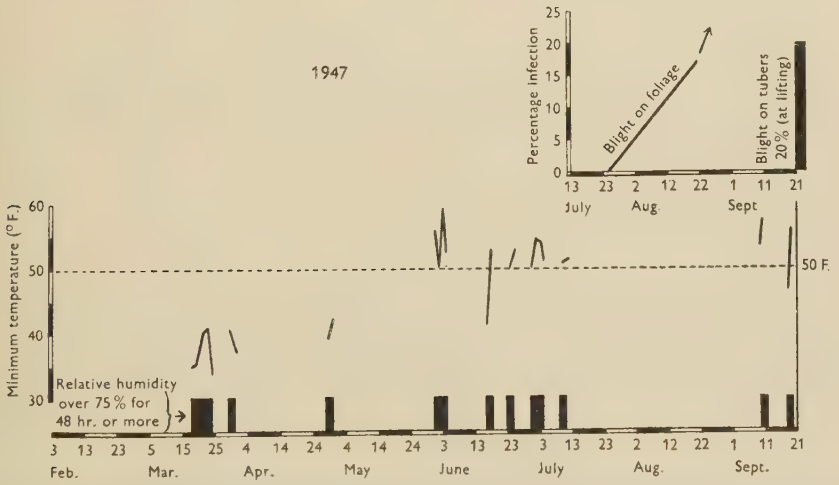


Fig. 2. For general explanation, see legend to Fig. 1. The first suitable period in 1947 was 1–3 June—fifty days before Blight appeared (see inset graph to same horizontal scale). When Blight appeared on 23 July, it was preceded by a suitable period 9–11 July. Auchincruive.

The graph for 1947 (Fig. 2), however, shows that conditions suitable for an outbreak of Blight were first experienced from 1 to 4 June. Blight did not appear until fifty days later, which is rather too long for a valid forecast. When Blight did appear, however, it was preceded by a suitable period from the 9 to 11 July—twelve days before the first appearance. The year 1944 was similar. Blight, therefore, does not always follow within a reasonable time of the first suitable climatic condition of the growing season, but when it does appear, it is heralded by the occurrence of a suitable period 12 to 16 days beforehand.

Auchincruive results might be generally typical of the central area of Ayrshire, but Blight appears about a fortnight earlier in Dumfries, and about fourteen days later in west Perth. With the collaboration of Mr MacDonald, Superintendent of the gardens at the Crichton Royal Infirmary, Dumfries, and Mr R. Rowat, County Organizer for Dumfriesshire, it has been possible to study the effect of climate on the appearance of Blight in this southern area for two years. Blight appeared on 28 June in 1948, and this first appearance was preceded by a suitable period on 14–15 June. The phenology of both events was similar, within three or four days, in 1947. During 1948, Sir James Denby Roberts of Strathallan Castle, Auchterarder, Perthshire, also collaborated in the investigation. He has installed the most reliable recording apparatus for this type of problem—a wet-and-dry-bulb, mercury-in-steel recorder, with the bulbs screened and continuously aspirated by means of an electric fan. Blight appeared at Strathallan on 22 July; the suitable climatic period occurred 11–13 July—eleven days before. These determinations in other districts have not yet been in progress for a sufficiently long period to warrant any generalizations. They do, however, reinforce the observation that Blight appears about the end of June in Dumfries and towards the end of July in west Perthshire. They also confirm that the first appearance of *P. infestans* is preceded by a suitable climatic period.

The use of Blight forecasts for protective sprayings

Protective sprays might be applied for one or more of the following reasons:

(1) For the control of an early attack of Blight with a view to increasing the yield of tubers.

(2) For controlling a late attack of Blight, to prevent its spread to the tubers at lifting time.

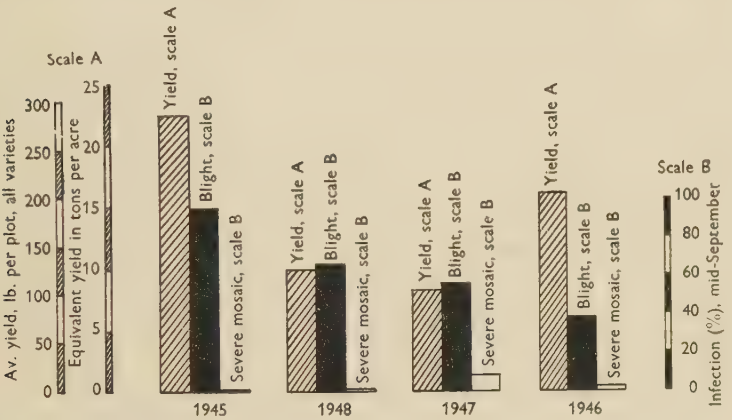
(3) To control an attack of Blight for size control in seed potato production.

Control of Blight for the last two reasons can also be accomplished by a 'destructive' spray, where the foliage is destroyed by sulphuric acid, sodium chlorate or other haulm-killing agent. Some aspects of these uses will now be discussed, as they affect the area typified by Auchincruive.

The effect of Blight on yield in south-west Scotland

Doncaster & Gregory (1948) have shown that three-quarters of the yield of potatoes has been made by the middle of August in England. General indications suggest that this would also be true in the west of Scotland.

It therefore seems important to inquire into the effect of Blight on yield in this area. Records of total yield of all varieties grown on the disease phenology plots do not offer any strong correlation with the amount of Blight (Fig. 3), nor is there any better evidence from individual varieties



Rainfall (in.)	1945	1948	1947	1946
June	3·72	4·90	2·62	1·95
July	2·67	2·59	2·99	3·33
Aug.	2·02	5·42	0·33	2·05
Sept.	4·32	4·75	4·60	5·12
4 months	12·73	17·66	10·54	12·45

Fig. 3. Four years' records of yield, percentage of Blight on the foliage in mid-September, and percentage of Rugose Mosaic in the stock, with figures for rainfall, Auchincruive, average of all varieties (21 plots), arranged in descending order of Blight severity—the same orders as Figs. 4 and 5, which refer to the same material.

(Table 1). The varieties Gladstone and Dunbar Rover have six plots in each year. Varying amounts of blight on the separate plots have been recorded in September in each year, so that it is possible to obtain, on an adequate scale, correlation coefficients (r, Table 2) between yield and Blight for these varieties. These are all very low, and show a virtual absence of correlation. Values for r are not even always negative, as would be expected if high yield depended on low amounts of Blight. Figures for the

Table 1. *Blight on foliage and yield of potato varieties*

Variety	1945 Blight on foliage 7 Sept. (%)	1945 Av. yield (lb. per plot)	1946 Blight on foliage 20 Sept. (%)	1946 Av. yield (lb. per plot)	1947 Blight on foliage 15 Sept. (%)	1947 Av. yield (lb. per plot)	1948 Blight on foliage 1 Sept. (%)	1948 Av. yield (lb. per plot)
Gladstone	90	282	30	208	51	95	35	127
Dunbar Rover	91	245	35	169	58	97	34	97
Kerr's Pink	97	348	25	246	33	127	2·7	156
Arran Pilot	90	200	93	164	99	91	95	107
King Edward	97	367	25	243	57	109	37	156

Figures for yield and Blight are the averages of three plots in each year for Kerr's Pink, Arran Pilot and King Edward, and of six plots for Gladstone and Dunbar Rover.

Table 2. *Lack of correlation between yield and the amount of Blight*

	Variety Gladstone		Variety Dunbar Rover	
	Correlation coefficient *r*	Probability *p*	Correlation coefficient *r*	Probability *p*
1945	−0·0356	>0·9	+0·083	0·9
1946	+0·506	0·3	−0·172	0·7
1947	−0·077	0·9	+0·034	>0·9
1948	+0·137	0·8	+0·217	0·7

probability of validity of the correlations (*p*, Table 2) are all so high, that in comparison with the usually accepted limit of 0·05, they indicate a complete lack of significance.

Fluctuations in overall yield do not appear to depend in major degree upon either the amount of foliage Blight at a particular time, the rainfall of June to September, or the amount of severe mosaic (Fig. 3). Perhaps all three factors are jointly involved, but such year-to-year correlations may not be adequate.

Relation between Blight on foliage and Blight on tubers

The four years 1945–8, for which detailed and standardized records are available, have fortunately included considerable variation in the rate of development of Blight on the foliage. Fig. 4 shows that early severity is followed by a relatively light amount of the disease on the tubers (1945, Fig. 4) and vice versa (1946, Fig. 4). No spraying for haulm destruction is

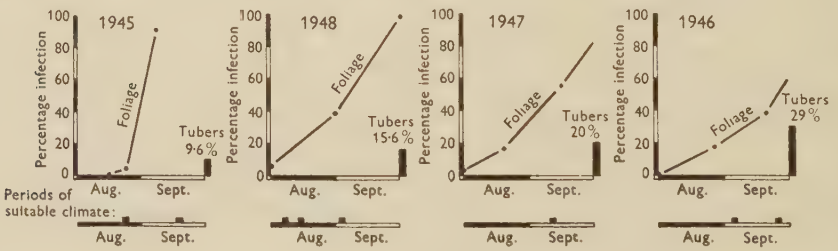


Fig. 4. Records over four years arranged in ascending order of the amount of Blight on the tubers at lifting time (shown in histogram form at the bottom right-hand corner of each graph). This also brings the curves giving the amount of Blight on the foliage into a descending order of steepness, showing that the faster Blight develops on the leaves, the less likely it is to produce tuber infection, and vice versa. Periods of suitable climate (Beaumont, 1947) are shown below the graphs. Auchincruive; averages of all twenty-one plots in each year. See also Figs. 3 and 5, which refer to the same material.

practised on the disease phenology plots, but the fungus evidently acted as its own haulm-killing agent in 1945. The inference is that protective sprays applied early in this year would have kept the haulm green for a longer period. This would doubtless have resulted in a heavier infection on the tubers, unless a destructive spray were given. Further investigation is necessary.

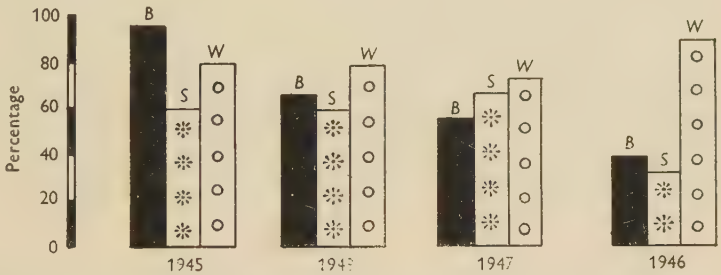
Protective sprays in years of slow Blight development do, however,

reduce the amount of Blight on the tubers, even in the absence of a destructive spray. Part of a crop of King Edward potatoes at Auchincruive was sprayed with Bordeaux Mixture on 9 August 1946 (cf. Fig. 4 for comparable Blight development). This application resulted in a decrease of 15 % of blighted tubers, from 26 % (unsprayed) to 11 % (sprayed).

Prevention of spread to the tubers can be accomplished more readily and certainly by destructive, than by protective sprays. Sodium chlorate (1·2 %) applied to the foliage on 7 September 1948 resulted in a decrease of Blight on the tubers from 17 % (unsprayed) to 9 % (sprayed). Large-scale applications of this substance coupled with earlier protective sprayings, however, result in less than 1 % of Blight on the tubers, when untreated crops give 10 to 30 %, according to the season. While the major control of spread to the tubers should be by destructive spray, the use of protective sprays, based on forecasts, would provide, in most years, a useful auxiliary treatment, and an insurance in case of unsuitable weather for the destructive spray.

The effect of Blight on seed and ware potatoes

Although Blight has not yet been shown to affect the overall yield in west Scotland it can affect the ratio between tubers of seed and ware sizes. Fig. 5 shows that when Blight on the foliage develops slowly as in 1946



	1945	1947	1946
Percentage Blight on:			
Seed	9·8	19·7	34·7
Ware	9·4	18·7	28·2
Significance of difference between percentage blight on seed and ware	p = between 0·7 and 0·6	p = 0·1	p = between 0·01 and 0·001	p = between 0·01 and 0·001
	Not significant		Significant	

Fig. 5. Percentages of Blight on the foliage in mid-September (*B*), and of seed tubers (*S*) and ware (*W*) in the total produce. Arranged in the same order as Figs. 3 and 4, which refer to the same material. Auchincruive.

(cf. also Fig. 4) there is a larger percentage of ware and a smaller percentage of seed. The percentages of these two sizes approached more nearly to equality in the other three years, when the amounts of foliage Blight were higher. Comparisons of the disparity between percentages of seed and ware are very highly significant between 1946 and 1947 (by *t*-test, p is far below 0·001) and between 1946 and 1945 (p is also far below 0·001). The longer period of green foliage in 1946 obviously converted more of the seed-size tubers into ware. Protective sprays used for tuber-size control are,

however, generally directed towards increasing the percentage of seed, and not of increasing the ware. A destructive spray is given before the amount of ware has begun to increase. Timing of this spray would depend on a grower's own observation, and there is no necessity for a forecast.

It has been noticed in all years that there is a tendency for the smaller sized tubers to have a slightly higher infection with Blight than the larger ones. Average figures for the twenty-one plots in each year are given below the histograms in Fig. 5. Differences are not significant when the amount of Blight on the tubers is relatively low (1945 and 1948). The differences are, however, significant when the amounts of Blight on the tubers are higher (1946 and 1947, Fig. 4). The greatest actual difference (6·5 %) is in 1946, with the greatest amount of Blight on the tubers. Reasons for this are not yet fully understood, but the results may either indicate a difference of relative position between seed and ware tubers, or a difference between their nutrition or nutrient content.

The use of Blight forecasts for destructive sprays

Crops of the early variety Epicure grown for seed on the coastal belt of Ayrshire reach the end of their period of effective growth about the time when Blight appears in the district. Should the disease have become established on the foliage at lifting time, it results in a heavy infection of the tubers, and heavy financial loss on storage. The remedy is to give a destructive spray *just before Blight appears*. There is usually no time to apply this fourteen days before lifting, as the opposed interests of farmer and merchant make the time of digging somewhat critical and often hurried. The provision of a forecast of the first appearance of Blight would introduce a precision, hitherto absent, into the timing of this important application.

Discussion

Blight does not always occur in the west of Scotland at a reasonable interval after the first suitable climatic period of the year. All outbreaks of the disease studied during the last five years have, however, been preceded by a suitable period ten to sixteen days before. Is it possible to find any formula which will separate the valid, from the non-valid periods of suitable climate? All the valid periods have occurred after the last days of June (26–28), and with the exception of 1948, all occurred after 1 July, which might therefore be called the 'zero time' of validity. Observance of this 'zero time' at Auchincruive would have allowed the issue of correct forecasts in all five years. It would also have avoided the utterance of false prophecies based on four non-valid periods in the two years 1944 and 1947. 'Zero time' for the Dumfries area would probably be earlier and for Stirling later, than the Auchincruive value. The conditions in each province must be assessed by measurement and experience.

Blight can attack the foliage of all common, susceptible commercial varieties of potato at any stage of shoot growth, if spores of *Phytophthora infestans* are present (Dr W. Black, Corstorphine, *in lit.*). Failure of Blight to follow suitable climatic periods before a 'zero time' cannot therefore be

ascribed to an unsuitable condition within the host. The most likely explanation is that the time of appearance of spores from an overwintering stage of the fungus depends on the general climate before the first outbreak. Beaumont's work on Blight (1947) was first carried out in Devon and Cornwall, where the average temperatures are 2–4° F. higher than those of

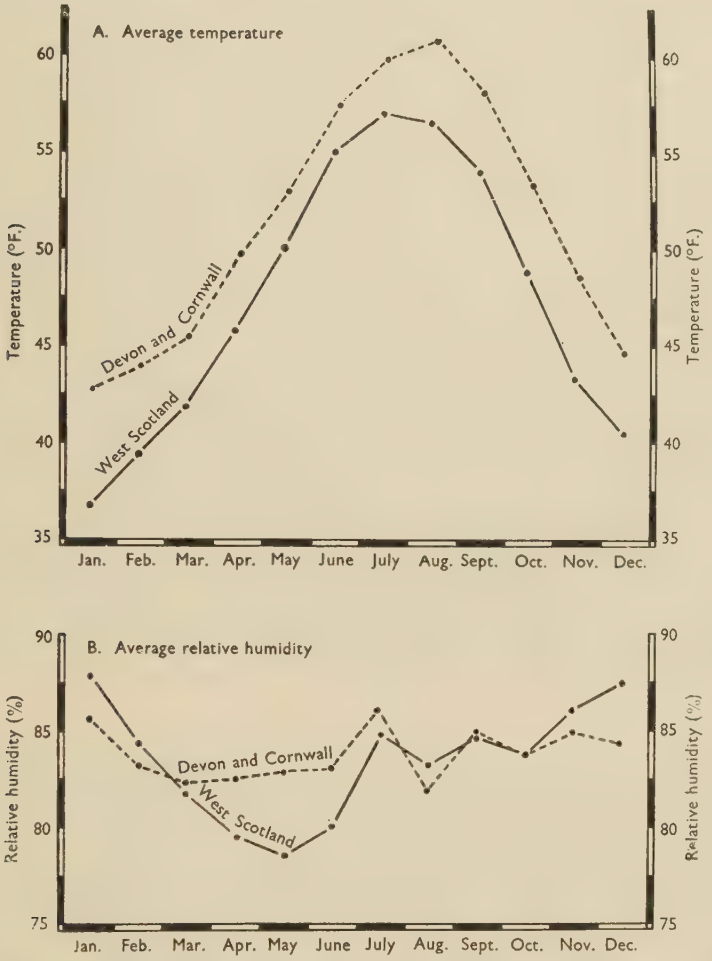


Fig. 6. Monthly temperatures and relative humidities averaged for Plymouth, the Lizard and Newquay in Devon and Cornwall: for Tiree, Rothesay, Renfrew, Auchincruive, Prestwick and Eskdalemuir in west Scotland. Figures for average relative humidity are calculated from the mean dew point and mean air temperature for the appropriate period, from records abstracted from the Air Ministry's Monthly Weather Reports, 1939–46.

west Scotland during the growing period, and are about 6° F. higher in January (Fig. 6A). There is also a higher average relative humidity in Devon and Cornwall during April, May and June (Fig. 6B). These facts may partly explain the early appearance of Blight in the southern area. It is interesting to note, however, that Beaumont (1947) also recorded

occasional suitable climatic periods early in the year which were not followed by Blight within a reasonable period.

It now seems fairly easy to forecast attacks of Blight, but is less easy to decide upon subsequent protective spraying action in the west of Scotland. Precise relations between such sprays and the overall yield still require determination. They can, however, at least be employed in tuber-size control and for the prevention of spread to the tubers. It is then reasonable to inquire if the *type* of Blight development on the foliage could be forecast. The years of quick development are those which have one or more suitable climatic periods in August (1945, 1948, Fig. 4). Absence of such suitable periods slows down the development of Blight on the leaves (1946, 1947, Fig. 4). Practical use could probably be made of announcements of the occurrence of suitable climatic periods in August, although the greatest need to apply protective sprays arises in years of slow Blight development when such periods are *not* recorded during August (see above). The last-mentioned type of prophecy would be difficult to organize. A useful forecast could hardly be made until the end of the month, by which time a grower would see the amount of Blight on his crops.

The present paper has tended to emphasize the importance of a destructive spray at the end of the growing season as a major element in the control of Blight. This does not mean that protective sprays are necessarily of limited use in the west of Scotland. They still play a part in the prevention of spread from foliage to tuber and are useful in seed-size control. Some years, indeed, have unsuitable climate for the application of destructive sprays, and the earlier application of protective sprays would then have provided a useful insurance against spread to the tubers. Protective sprays would, moreover, reduce the level of tuber disease in most years. Forecasts of the first appearance of Blight and probably of subsequent periods of suitable climate would therefore be useful.

Summary

The validity of forecasts of the first appearance of Potato Blight based on Beaumont's (1947) temperature-humidity rule has been investigated for the west of Scotland. Forecasts were all valid after the last days of June for the five years from 1944 to 1948, and were not valid before that time. This period is suggested as the 'zero time' of validity of forecasts in Ayrshire, and available evidence suggests that it varies for different districts.

The effect of foliage Blight upon overall yield cannot yet be specified with accuracy. Forecasts would, nevertheless, be useful for protective sprays used to diminish the spread of Blight to the tubers. Such sprays should, nevertheless, always be used in conjunction with destructive sprays when possible. Reliable prophesies of the first appearance of Blight would also be valuable for timing the application of destructive sprays in the special case of seed crops of Epicure potatoes in the early districts of Ayrshire.

The amount of Blight appearing naturally on the tubers is greatest when the disease develops slowly on the foliage. Such years may also bring a greater proportion of ware to seed, and there is a tendency for more Blight to appear on seed-size tubers than on the ware. Years of quick

development of *Phytophthora* on the leaves have relatively little Blight on the tubers when no control of the disease is attempted.

The valued assistance of Mr D. McAughtrie in obtaining the records of Blight on the tubers and for his general care of the disease phenology plots is acknowledged with pleasure. Mr Iain D. L. Scott kindly performed the laborious calculations of average temperature and of average relative humidity shown in Fig. 6. Other members of the staff of the Plant Pathology laboratory have also given valuable help.

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Postscript—February 1950

Further work during 1949 suggests that the loss in overall yield from Blight on the foliage may be as much as 25 % of the possible crop in years of early development of the disease. The reasons why no correlation is demonstrable by the methods used in this paper appear to be (1) the yields have fluctuated in some years by more than 25 %, due to other causes, and (2) there is no yield of Blight-free potato plants for comparison in any one year.

NOTE ON A METHOD OF INDUCING SPORANGIUM FORMATION IN *PYTHIUM UNDULATUM* PETERSEN, AND IN SPECIES OF *SAPROLEGNIA*

By E. K. GOLDIE SMITH

Methods of promoting the formation of sporangia in species of *Pythium* are based on the principle that sporangia are formed when well-nourished mycelium is transferred to water, from a rich nutrient medium such as lima bean agar (Drechsler, 1941, 1946), or pea broth (Middleton, 1943). According to Middleton (1943, p. 10), species which do not readily form sporangia may be encouraged to do so, by adding small amounts of soil or of organic matter. Drechsler (1946, p. 783) has emphasized that the water should be deep enough just to cover the medium sparingly, and that, in order that 'the water may spread out thinly and not collect in thick unmanageable pools', the floor of the Petri dish 'should be freed of all greasy film by scouring it thoroughly with an abrasive cleanser'. While continual irrigation is necessary for zoospore formation in most species according to Middleton, Drechsler (1946, p. 824) has found that occasional renewal of the water suffices to keep up the process in *P. vexans* for several days.

During a study at Royal Holloway College of a fungus isolated from the leaves of water-lilies in a pond in the grounds and identified as *Pythium undulatum* Petersen, difficulty was experienced in obtaining sporangia in any great quantity. The following method was therefore devised to promote their formation. It has been tested at frequent intervals since the study was begun in the autumn of 1946, and has always been found reliable, at temperatures of 16–24° C.

A rich nutrient agar, such as cornmeal agar, is poured so that it just covers the bottom of a Petri dish. A sterile glass ring is placed in it and inoculated in the centre, so as to restrict the development of aerobic bacteria which may be present, according to the method described by Raper (1937). It has not been found necessary to raise the ring from the bottom of the dish by means of glass 'feet'. When a good growth has developed outside the ring, usually on the second day after inoculation, halved hemp seeds, previously sterilized by boiling for five minutes, are placed with the cut surface downwards on the margin of the mycelium. After a period of time up to twenty-four hours, the half seeds are transferred to a Petri dish of sterile distilled water of such a depth that they are barely covered. Not more than three half seeds are put into any one dish. If the seeds are left longer in contact with the mycelium on agar, vegetative growth is found to predominate over sporangium formation when they are transferred to water, and although several changes of water at intervals of a few hours may then promote the formation of sporangia, these are usually of only moderate size and activity. While good results have been obtained with seeds left on the agar for only four hours, a period of ten to twenty hours has been found

most satisfactory. Sporangia appear after a few hours in water, and after fifteen to twenty hours the seeds are usually found to be bristling with stout sporangiophores bearing large ellipsoid sporangia which are actively discharging, and being vigorously renewed by multiple internal proliferation. This spontaneous activity usually lasts for many hours, and its longer continuance may be promoted by changing the water several times during the day. As the culture ages, secondary sporangia are formed by subsporangial branching rather than by internal proliferation, and the lengthening sporangiophore also branches in a manner apparently unrelated to the primary sporangium at its tip. As the vigour of the mycelium declines, or as staling-products are allowed to accumulate, the later-formed sporangia show a decrease in size, and a change from an ellipsoid to an ovoid shape. Finally, four or five days after the transfer to water, the large central vacuole of dormancy is found to have developed in the sporangia, which respond to a change of water, not by discharging zoospores, but by growing out into a hypha.

A slight modification of this method may be used for species of *Saprolegnia*, in which a vigorous crop of active sporangia can be obtained three or four days after transfer of the seeds to water. The discharge of a large number of sporangia at a particular time can be relied upon if the water is changed several times on the first two days, and the cultures are then left completely undisturbed until the fourth day. Discharge of many of the sporangia which have been forming during this period may then be induced by a change of water half to one hour before the cultures are required. This method has been tested on species of the diclina and ferax groups of *Saprolegnia*, and has been used with great success in the preparation of material for class work at Royal Holloway College, and, subsequently, at the University of North Carolina, Chapel Hill, N.C., U.S.A., where further work on sporangium formation and discharge in Saprolegniaceae is now in progress.

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ON A THERMOPHILE SPECIES OF *CHAETOMIUM*

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(With Plates 4–7 and 9 Text-figures)

Fungi which are known to be able to grow and reproduce themselves at temperatures between 40 and 50° C. form a very small though heterogeneous group. Collectively they are termed thermophiles. But there are some which, although they thrive best at these high temperatures, are able to thrive also quite well in the lower temperature range common to the majority of fungi, i.e. approx. 15–30° C; these are called psychrotolerant. Others thrive best within the lower temperature range but are able to grow also at much higher temperatures; they are called thermotolerant. The remainder are able to perform their vital activities normally only within the upper temperature range, i.e. approx. 30–55° C.; these are the true thermophiles, and only four have so far been recorded. They are: *Thermomyces lanuginosus* Tsilinsky, *Thermoidium sulphureum* Miehe, *Thermoascus aurantiacus* Miehe, and *Anixia spadicea* Fuckel. Their cardinal temperatures for normal growth* have been recorded by Miehe (1907, 1910) and Noack (1912) as follows:

Thermomyces lanuginosus: min. 30° C.; opt. 40–57° C.; max. 60° C.

Thermoidium sulphureum: min. 29–30° C.; opt. 35–45° C.; max. 53° (or 55° C.)

Thermoascus aurantiacus: min. 35° C.; opt. 40–46° C.; max. 55° C.

Anixia spadicea: min. approx. 30° C.; opt. approx. 45–46° C.; max. approx. 58° C.

In a preliminary note (La Touche, 1948) I recorded under the designation M.R.A. 112, a 'thermophile ascomycete producing perithecia which in many respects resemble those of a *Chaetomium*'. Further study of this fungus has shown that it is in fact a hitherto undescribed species of *Chaetomium*. Of the four true thermophile fungi already recorded, two of them, *Thermoascus aurantiacus* and *Anixia spadicea* are ascomycetes. This new thermophile, for which I propose the name *Chaetomium thermophile* n.sp., is therefore the third true thermophile ascomycete on record.

Description

Chaetomium thermophile n.sp.

Hyphae septate, branched, at first hyaline with thin walls, then olivaceous or brownish olive with thicker walls, 2–8μ wide, in parts dilated. Perithecia superficial, more or less gregarious, globose or subglobose, 75–172μ

* Noack (1912) recorded some abnormal growth form *Thermomyces lanuginosus* at 25·5° C., for *Thermoidium sulphureum* at 24° C., and for *Thermoascus aurantiacus* at 27° C. Tsilinsky (1899) recorded abnormal growth for *Thermomyces lanuginosus* between 60 and 63° C.

diameter, at first lightly coloured then dark olivaceous or brownish olive, parenchymatous in texture, made up of polygonal cells; ostiole terminal, surrounded by thin-walled hyaline cells; basal hyphae olivaceous or brownish olive, branched or unbranched, sometimes forming a thick strand; lateral hairs continuous or septate, unbranched or branched at the tip, hyaline or scarcely coloured; minute granules distributed over the surface; terminal hairs, unbranched or scarcely branched at first, much branched dichotomously and densely entangled when mature, unequally thickened and constricted along their length, tortuous, ampulliform or subampulliform at the base, covered with minute granules, olivaceous or brownish olive, septate, 2–4μ wide, branches and branchlets often reflexed and anastomosing, terminal branchlets diverging at an obtuse angle, with hyaline tips. Asci linear cylindrical or rarely club-shaped, short stalked, hyaline, 50–57 × 7–8μ, pars sporif. 44–54μ, evanescent, eight-spored. Ascospores unistichous or rarely irregularly distributed, dark olivaceous or brownish olive when mature, globose or irregularly subglobose, with a single apiculus; 7–8μ diameter or occasionally 9 × 7μ, when seen edgewise 5–6μ wide.

Habitat on fermenting wheat straw in the laboratory.

A specimen of M.R.A. 112 which is to be regarded as the type has been deposited in the Herbarium of the Commonwealth Mycological Institute.

Diagnosis

Chaetomium thermophile n.sp.

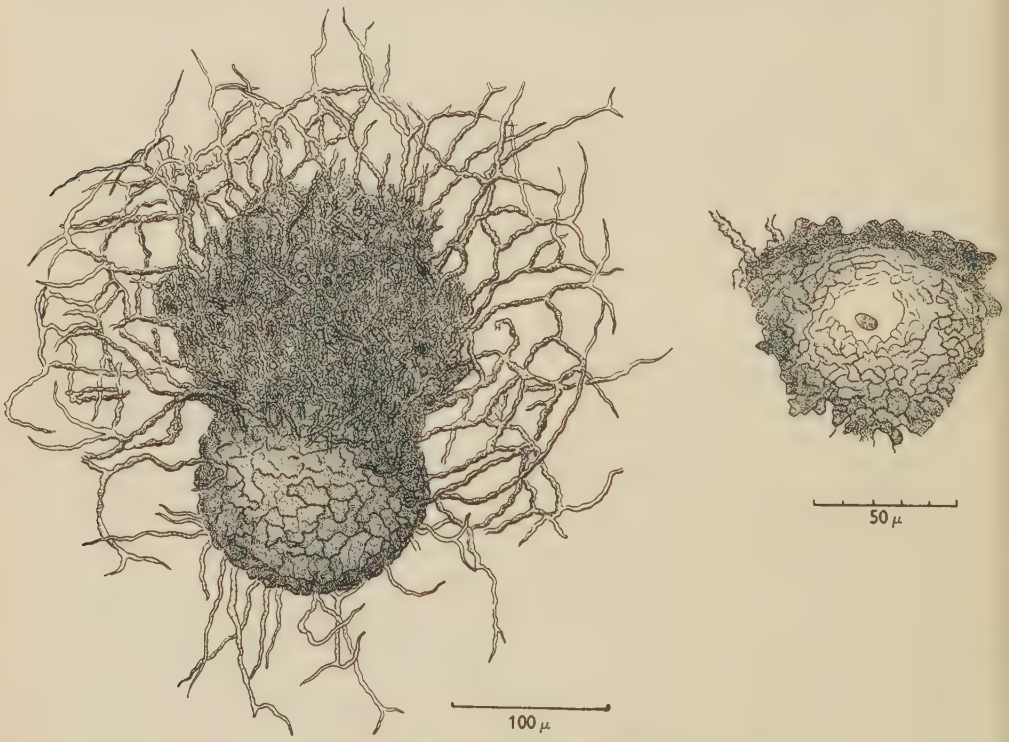
Hyphae septatae, ramosae, primo hyalinae parietibus tenuibus, demum olivaceae vel brunneo-olivaceae parietibus crassioribus, 2–8μ latae, passim inflatae. Perithecia superficialia, plus minusve gregaria, globosa vel subglobosa, 75–172μ diam., primo dilute colorata dein fusco olivacea vel brunneo-olivacea, contextu parenchymatico e cellulis polygoniis composito; ostiolum terminale, cellulis circumjacentibus hyalinis, tenuiter tunicatis; hyphae basales olivaceae vel brunneo-olivaceae, simplices vel ramosae, interdum in funiculum crassum congregatae; pili laterales continui vel septati, simplices vel apice ramosi, hyalini vel deorsum dilute colorati, externe minute granuloso—incrustati; pili terminales primo non vel vix ramosi sed maturitate copiose dichotomo-ramosi et dense intricati, irregulares, nunc angustati nunc inflati, basi ampulliformes vel subampulliformes, tortuosi, minute granuloso-incrustati, olivacei vel brunneo-olivacei, septati, 2–4μ lati, ramis ramulisque saepe reflexis et anastomosantibus, angulo obtuso divergentibus, apicibus hyalinis. Asci anguste cylindracei vel raro clavati, breviter pedicellati, hyalini, 50–57 × 7–8μ, pars sporif. 44–54μ, evanescentes, octospori. Ascosporae unistichae vel raro irregulariter dispositae, maturitate fusco-olivaceae vel brunneo-olivaceae, globosae vel irregulariter subglobosae, uni-apiculatae, 7–8μ diam. vel aliquando 9 × 7μ, e latere visae 5–6μ latae.

Hab. in stramento triticino fermentescente, in laboratorio.

Occurrence

Although I found *C. thermophile* M.R.A. 112, originally on the straw of artificial composts while these were being maintained in incubators at

elevated temperatures, I have since obtained other apparently similar specimens quite frequently on straw itself which was fermenting at temperatures between 40 and 50° C. It was necessary only to add a little water to the straw in a beaker with a Petri dish lid to cover it, and then to place the beaker in an incubator at about 45° C. Usually, under these conditions, there developed, within two or three days, a copious growth of such moulds as *Mucor pusillus* Lindt, *Aspergillus fumigatus* Fres., *Thermomyces lanuginosus* Tsilinsky, and also what seems to be a species of *Monotospora*. After three or four days the perithecia of *Chaetomium thermophile* could be discerned with the aid of a pocket lens 10 ×. They were sometimes difficult to find on account of the dense growth of other moulds especially *Thermomyces lanuginosus* and the Monotospora-like mould, both of which produced spores in such large quantities that they often masked the young perithecia.



Text-fig. 1. Mature perithecium. Inset: ostiole of perithecium.

Material and methods

Isolation in pure culture

Originally, isolation was a matter of some difficulty, especially from bacteria, but by washing the material in sterile water acidulated with a little hydrochloric acid and making successive transfers from the periphery of agar cultures, I was able to obtain eventually a pure culture which I labelled M.R.A. 112. With the more recent isolations (M.R.A. 239, 275, 283),

which were made from fermenting straw, I encountered much less difficulty by using potato-carrot extract agar according to the formula given by Langeron (1945). The reaction of this medium was adjusted to pH 4 and Rose Bengal was added to it in the concentration of 0·0067 % of the total fluid. This medium effectively reduced contamination by bacteria, and moulds such as *Aspergillus fumigatus* and *Mucor pusillus* could be avoided or checked by incubating the transfer culture at 55° C.

Isolation of single sporelings

For this purpose a suspension of ascospores in potato-carrot extract pH 5·5 was made and placed in the incubator at 45° C. When the spores had germinated, drops of the suspension were pipetted on to the surface of filtered potato-carrot extract agar and the sporelings isolated by drawing out the drops in different directions with a fine bead at the end of a glass rod. Cultures derived from a single sporeling isolated in this manner and labelled M.R.A. 112 have provided the material for the description and diagnosis of this species as well as for the experiments on its growth at different temperatures. Cultures from M.R.A. 239 were also derived from a single sporeling isolated in this manner.

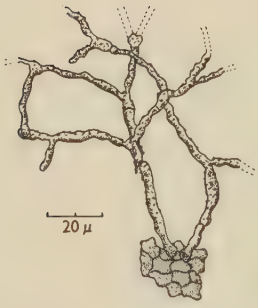
Culture media

Pure cultures were at first stored and propagated on 3 % malt extract agar pH 5·5, but this medium was eventually abandoned for routine work, as, with time, production of perithecia as well as vegetative growth fell off considerably. Potato-carrot extract agar pH 5·5 has been a good medium for the production of perithecia and for storage purposes, but, probably owing to the variable nutritive content of different batches, production of perithecia, although generally very good, was at times erratic. Straw extract agar was made by boiling straw powder in water (50 g. straw powder in 1 l. of distilled water) for thirty minutes, filtering, adjusting to pH 5·5 and adding 30 g. of agar. This was not a good medium as it supported only a thin sterile growth and its use was therefore discontinued. Potato plugs and carrot wedges sterilized in test-tubes with a little water proved to be excellent for both vegetative growth and production of perithecia. Straw, cut up into lengths of about 2½ in. and sterilized in 100 ml. flasks with a little water (distilled or tap water) was very good for the production of perithecia, although results were somewhat variable. As this was the material from which the fungus was frequently isolated it was used as the medium of choice for providing mycelium and perithecia, etc., for the description and diagnosis, and cultures grown on it were studied more particularly than on the other media. All these media were sterilized at 120° C. and 15 lb. pressure for twenty minutes.

Conditions of culture on agar media

It was obvious that cultures could not be maintained at the high temperatures to which they were exposed in the incubators unless a high relative humidity could also be provided. Accordingly, various devices

were tried with a view to providing a saturated atmosphere. For test-tube slopes the best arrangement consisted of a capsule made up with two beakers in the following manner: a 250 ml. beaker was placed upright on the bench; test-tubes containing inoculated agar slopes were wrapped in a large sheet of filter-paper so as completely to include the cotton-wool plugs, and then stood upright in the beaker; water of the required temperature was poured into the beaker until it was almost filled, and finally a second beaker of the same capacity was inverted over the test-tubes and pushed down until its rim was in contact with the rim of the upright beaker. The same result could be achieved on a smaller scale by placing each test-tube within an inverted boiling-tube lined with filter-paper and supporting it in a beaker containing water of the required temperature, in either case a beaker containing water in which a sheet of filter-paper was partly immersed was also placed in the incubator. For Petri dish cultures the best method of ensuring a saturated atmosphere was to enclose the cultures between two plastic bowls containing some water and filter-paper.



Text-fig. 2. Basal part of terminal hairs from mature perithecium.

Text-fig. 3. Peripheral part of terminal hairs of mature perithecium.

Description of cultures

Wheat straw in Erlenmeyer flasks

At 45° C. Within twenty-four hours mycelium grew out extensively from the inoculum (spores or mycelium), appearing white or colourless, along the surface of the straw, and eventually it reached the surface of the liquid at the bottom of the flask. By the end of the second day young perithecial stages began to appear as minute white knots on the mycelium. By the third day they became light greenish grey, Artemesia Green (R.)* to Lily Green (R.). On the fourth day they were brown, Fuscous (R.) or Fuscous Black (R.). At first they were discrete in distribution but as they became more numerous they formed felt-like patches in which they were individually more or less indistinguishable under the pocket lens 10 ×. Often, they were

* Ridgway: "Color Standards and Color Nomenclature."

more or less overgrown by a sparse colourless mycelium. Perithecia, containing mature spores, were found usually on the third day but exceptionally after forty-eight hours, (M.R.A. 239); and Fuscous (R.) columnar cirri were produced by the fifth day. Sometimes the perithecia developed in zones on the lengths of straw, more especially again in the case of M.R.A. 239. (Pl. 5, fig. 2). Production of perithecia was somewhat erratic as regards numbers for reasons which were not evident, and there was considerable variation even between different cultures of the same strain grown at the same time.

At 55° C. Mycelium was more abundant than at 45° C. but no perithecia were developed by the end of three weeks.

At 35° C. Growth was very slow, and at the end of three weeks only a few perithecia had developed and the olive brown hyphae which were closely septate were also much dilated in parts.

Slopes of 3 % *malt extract agar*, pH 5·5 *at* 45° C.

M.R.A. 112. Turf at first colourless. In three or four days centre dense, felt-like, wrinkled, Greyish Olive (R.), with superficial sparse fine white mycelium; margin wet, cream-coloured. In two weeks becoming Dark Olive (R.). Reverse black. Perithecia: at first in various shades of greenish-grey, becoming eventually Fuscous (R.).

M.R.A. 239. Turf at first colourless. In three or four days, centre close-velvety, not wrinkled, Dark Greyish Olive (R.) merging outwards into Mouse Grey (R.); margin wet Cream-coloured (R.). Growth more vigorous than M.R.A. 112. Perithecia: same as M.R.A. 112 but more abundant.

Slopes of potato-carrot agar pH 5·5 (*P.C.A.*) at 45° C.

M.R.A. 112. White to colourless, sparse. Perithecia sometimes on second day, light greenish grey, Vetiver Green (R.) and Sage Green (R.); within three days already dark brown, Dark Fuscous Black (R.) in some cultures; tendency for perithecia to develop in zones.

M.R.A. 239. Same as M.R.A. 112, but perithecia more abundant and in more definite zones. (Pl. 5, fig. 4).

Carrot wedges in test-tubes at 45° C.

M.R.A. 112. By third day carrot wedges covered by dense growth of mycelium and perithecia; perithecia Deep Greyish Olive (R.) to Dark Olive Grey (R.).

M.R.A. 239. Same as M.R.A. 112 but perithecia more uniformly distributed.

Germination of Ascospores at 45° C. (Text-fig. 8)

Ascospores germinated within three hours in melted P.C. extract agar, pH 5·5, containing Rose Bengal added in the concentration of 0·0067 % as a precaution against bacteria. When they were examined at the end of three hours many exhibited a minute globule of protoplasm at the point where the apiculus is situated, showing that this is in fact a raised germ-pore.

At the end of four hours one or more short hyphae had grown out from the initial globule. At the end of six hours these hyphae showed branching.

Text-fig. 4. Early stage in development of perithecium. Unbranched hairs.

Text-fig. 5. Early stage in development of perithecium. Branched hairs showing ampulliform bases.

Cardinal temperatures for growth

Results so far obtained indicate that the maximum temperature at which growth visible to the naked eye can still occur lies at 60° C. or slightly above it. Inocula transferred suddenly from a lower temperature, e.g. 45° C.

to this level most frequently failed to show any growth, but when the transfer was made gradually some slight increase in growth occurred. In Table 1, the average linear growth along one diameter of three inocula at

Table 1

Total growth in cm. at end of	Control at 45° C. for 3 days		Experimental: At 58–59° C. for 4 days		Experimental: At 60–61° C. for 5 days		Experimental: At 61·5° C. for 5 days	
	Replicates	Average	Replicates	Average	Replicates	Average	Replicates	Average
2 days	4·6 4·3 4·8	4·6	0·6 0·3 0·7	0·5	Not measured		0·15 0·1 0·05	0·1
3 days	Slopes covered	7·0	0·7 0·45 1·1	0·8	1·0 0·95 1·05	1·0	Not measured	
4 days	—		0·95 0·75 1·35	1·0	Not measured		Not measured	
5 days	—		—		1·95 2·35 3·55	2·62	0·45 0·5 0·15	0·37

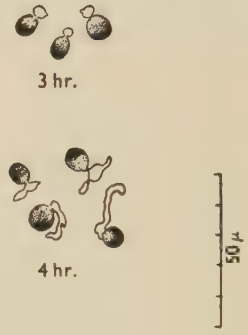
temperatures starting at 58–59° C. and increasing gradually to 61·5° C., is shown over an extended period and is compared with that of three inocula maintained at a temperature of 45° C. for the first three days. The material for this experiment consisted of disks measuring 4 mm. taken at a distance of 2 cm. from the centre of a three-day-old Petri dish culture grown at 45° C. in a saturated atmosphere. Eight slopes each containing originally 5 ml. of melted P.C.A., pH 5·5, were inoculated at about their centre with one of these disks and then set up in boiling tubes as previously described. They were then divided into two sets, each in a large beaker of water, one of which was placed in an incubator at 45° C. and the other at 58–59° C. Growth at these high temperatures (60–61·5° C.) tended to be submerged and was probably degenerate. Some earlier tests, using 3 % malt extract agar, pH 5·5, resulted in a small amount of moist creamy growth somewhat resembling a bacterial or yeast colony which when examined microscopically showed much disorganized hyphae. Such cultures when placed at 45° C. did not produce any further growth and could be presumed to have been killed after slight growth at 60–61·5° C. Other inocula on P.C.A., pH 5·5, which did not succeed in growing during several days' exposure to 60–61·5° C. showed visible growth after a further period of several days at 45° C. and thereafter appeared to grow quite normally and in fact produced abundant perithecia. There was thus a definitely prolonged lag in growth as a result of exposure to 60–61·5° C. for several days.

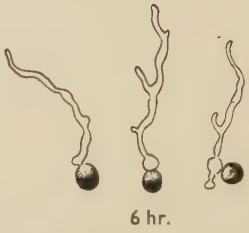
Results of growth experiments at more favourable temperatures, viz. 55, 50, 45 and 35° C., which will be published in a separate paper, indicate that the optimum growth lies around 50° C. and that the optimum range is between 40 and 55° C.

As regards the minimum temperature at which growth visible to the naked eye can still occur, this, from results so far obtained, is approximately 27° C.; thus when Czapek-Dox agar pH 5·5 was inoculated with disks of

Text-fig. 6. Young asci and asci containing immature spores.

Text-fig. 7. Mature ascospores.





Text-fig. 8. Germinating ascospores.

Text-fig. 9. Mature mycelium.

mycelium culture and exposed to 25° C. no growth was observable after a week at this temperature, but when the temperature was raised to 27° C. slight growth was observable after a further six days. An earlier record showed slight growth between 26 and 27° C. on 3 % malt extract, pH 5·5, of a wet creamy type resembling that obtained on this medium at temperatures near the upper extreme. No growth was observed at any time on any media at temperatures below 26° C.

Much work regarding the influence of temperature on this fungus still remains to be done, but from results obtained up to date the cardinal temperatures for its normal growth may be stated as being approximately the following: min. 30–35° C., opt. 50° C., max. 55–58° C. The absolute minimum temperature for growth visible to the naked eye on media so far used is 27° C. or slightly below it, and the absolute maximum for growth visible to the naked eye on these media is 61° C. or slightly above it.

Discussion

A full and critical discussion of the diagnostic features of this fungus and their taxonomic significance must await the isolation and examination of a large number of strains. Only then will it be possible to consider this fungus in its proper perspective. The discovery by me, on fermenting straw, with and without additions, of yet another thermophile *Chaetomium*, M.R.A. 272, also with cylindrical asci but differing from M.R.A. 112 in certain other respects, would suggest the advisability for this course. Several other strains, apparently morphologically similar to M.R.A. 112, have already been isolated by me but have not yet been critically examined.

There is, however, at least one structural feature in M.R.A. 112 which calls for immediate comment; this is the cylindrical shape of the asci, and it is not necessary to seek far into the literature to conclude that this is an exceptional feature in the genus *Chaetomium*; for example, the monograph by Chivers (1915) on the genus *Chaetomium* includes descriptions of some twenty-eight species of which only three, *C. simile* Massee & Salmon, *C. crispatum* Fuckel, and *C. tortile* Bainier, have cylindrical asci. Moreover, in passing, it may be pointed out that in all these three species the perithecial hairs are unbranched, whereas in M.R.A. 112 they are conspicuously branched. In M.R.A. 112 this point about the asci is made even more interesting because occasionally club-shaped asci may be found interspersed amongst the cylindrical ones.

Growth phenomena recorded in this paper must be considered with the reservation that the behaviour of this fungus has been up till now very erratic. Many cultures have died both at laboratory temperatures and at the experimental temperatures; this has been especially the case where the growth has been mainly vegetative Also prolonged culture on agar medium, especially malt extract agar, has considerably reduced the fertility of M.R.A. 112 on these media. This of course occurs commonly enough in culture work but appears to me to be exceptionally frequent in this case. Cultures on straw have been more satisfactory in this respect.

Although apparently a quite common fungus on fermenting straw with or without the addition of other substances, *C. thermophile* has been

overlooked; this is presumably owing to the exceptionally high temperature range within which it thrives. In this temperate climate it could grow only under exceptional conditions, i.e. where high temperatures prevail. Noack (1912) and Miehe (1907, 1910) both favoured the view that heaped up fermenting masses of vegetation enable thermophile organisms to propagate themselves, but Noack also considered that during the summer months short periods of insolation of the soil surface were a contributory factor in their survival. It is probably only a matter of time before *C. thermophile* will have been recorded under the conditions described by these authors.

My grateful thanks are due to: Miss E. Wakefield, Botanic Gardens, Kew, for her very constructive and generous help with the Latin diagnosis; Prof. J. Doyle, Botany Department, University College, Dublin, for granting me dark-room facilities in his laboratory and the services of his laboratory assistant Mr T. Dunn in order to take the photomicrographs; Dr R. L. Edwards, Director of Research, Mushroom Research Association, for devising the synthetic composts from which I first obtained this fungus, and for suggestions regarding growth data; my colleague Mr S. Burrows for his help in the photographing of M.R.A. 239 cultures; the staff of the Commonwealth Mycological Institute for their interest and help; the Directors of the Mushroom Research Association for their kind permission to publish this work, and finally, my assistant, Miss Barrows, whose very substantial help throughout this research made possible the publication of these results.

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EXPLANATION OF PLATES

Plate 4

Perithecia of *C. thermophile* n.sp. on straw epidermis from compost.

Plate 5

Fig. 1, Young perithecia, M.R.A. 112 on strip of straw epidermis. Magn. × 80 approx.
Fig. 2, Zones of perithecia M.R.A. 239 on straw. Reduced by one-half.
Fig. 3, Perithecium of M.R.A. 112 showing ostiole.
Fig. 4, P.C.A. cultures of M.R.A. 239 showing zoning of perithecia.

Plate 6

Fig. 1, Asci from perithecium of M.R.A. 112 containing young ascospores. Magn. × 270 approx. stained Chlorazol Black E.
Fig. 2, Ascus with irregularly arranged spores amongst asci with unistichous spores; from perithecium of M.R.A. 112. Magn. approx. × 300. Stained Chlorazol Black E.

Plate 7

Asci containing young ascospores M.R.A. 112. Magn. × 800 approx. Stained Chlorazol Black E.

(*Accepted for publication* 8 *April* 1949)

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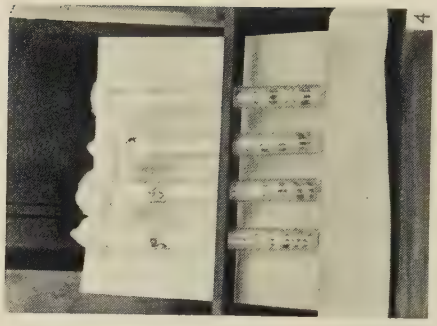
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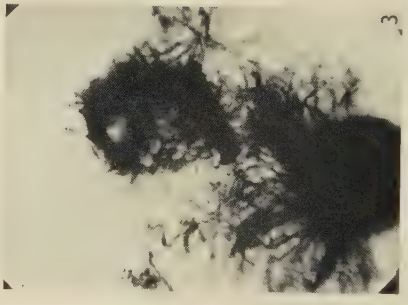
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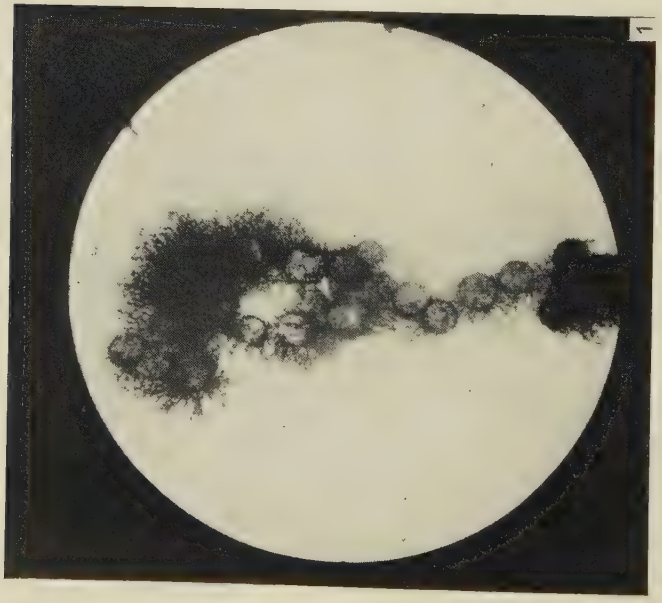
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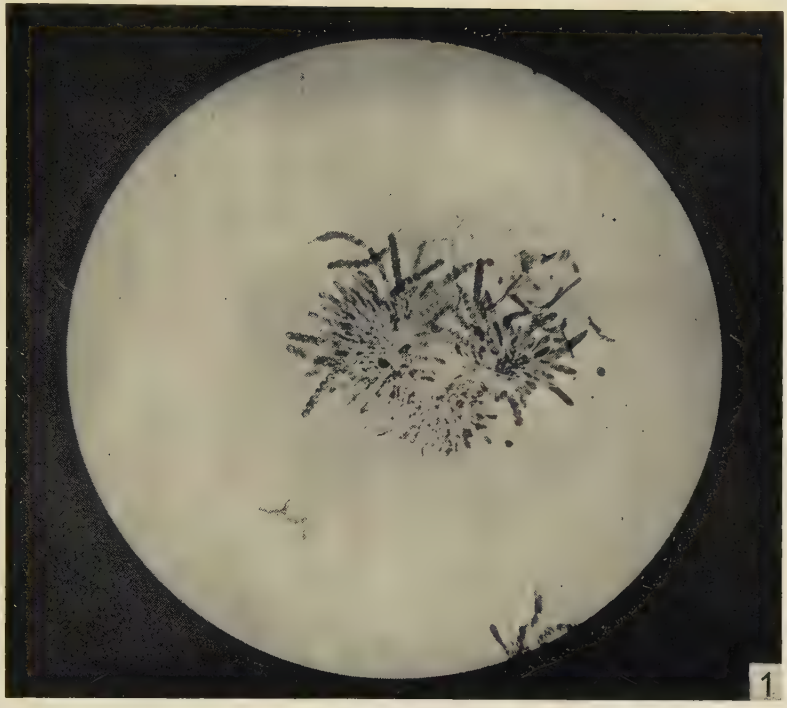
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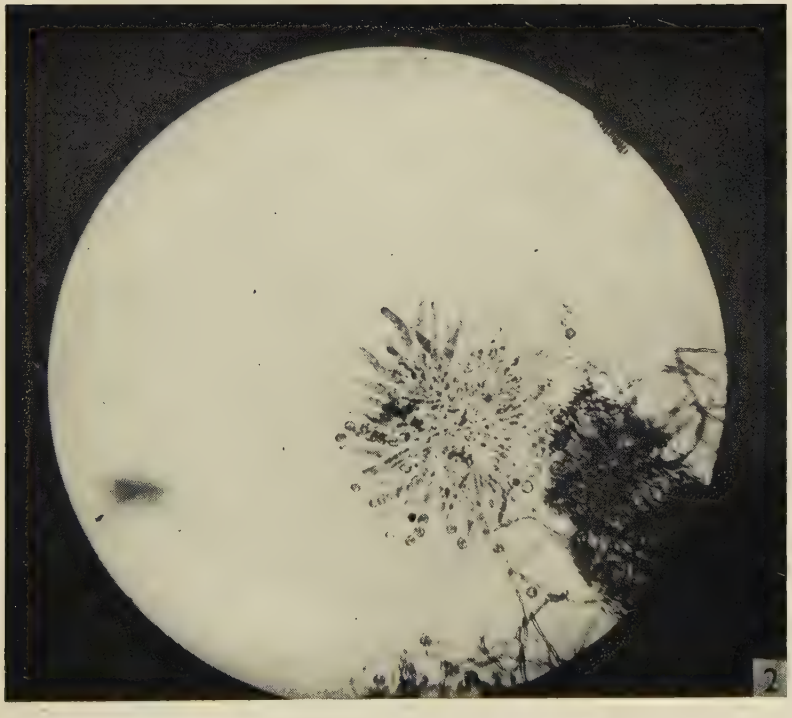
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A NOTE ON THE DISTRIBUTION OF *PORIA OBLIQUA* (PERS.) BRES.

By S. BATKO

(With Plate 8)

About eleven years ago Dr Findlay (Findlay, 1939; Cartwright & Findlay, 1946) collected near Aviemore in Scotland an interesting fungus on a birch tree. It was very brittle and of a charcoal-like appearance. No trace of a fertile layer was found on it. Further investigation and culture in the Laboratory at Princes Risborough have proved that the specimen collected belongs to *Poria obliqua* (*Xanthochrous obliquus*) Bourdot & Galzin. It has been recorded from Russia, Sweden, Yugoslavia and North America.

So far as I am aware no more specimens have been recorded in the British Isles since then. During my research work in the section of Mycology at Princes Risborough I was encouraged by Mr K. St G. Cartwright to give my attention to the distribution of that fungus in this country. In April 1948 I found one specimen of it on a birch (*Betula pubescens* Koch.) near Findogask (Perthshire), but no more specimens of it were found near Perth in spite of much searching around Findogask. It was found by me again in June and July 1948 on birches (*B. pubescens* Koch.) along the banks of the River Dee between Braemar and Inverey. It was rather common and often growing with other parasitic fungi, e.g. *Polyporus betulinus* Bull. and *Taphrina betulina* Rostr., etc. In February and April 1949 I found two specimens of it on the birches (*B. pubescens* Koch.) at Hallburn near Longtown, Cumberland. The first specimen is illustrated in Plate 8. It consists of several patches all breaking off the bark of the tree. The second specimen grew on a forked stem of a birch. The specimens from Cumberland are the first recorded in England. No fertile specimens have been found up to now in this country.

The general distribution of this interesting fungus needs still further investigation. An interesting note may be found in the German book (Neger, 1919, p. 231). He says about *Polyporus nigricans* Fr.: 'auf Birke sein häufigstes Substrat-werden in der Regel richtige Frucht Körper überhaupt nicht gebilded, sondern nur unförmliche, rissige, schwarze Knollen ohne Porenschicht.' That description and also the picture shown in the book rather suggest that the fungus belongs to the *Poria obliqua* group. I saw before the war, in the Herbarium of the Forestry Department in Lwów, a specimen corresponding to *P. obliqua*, labelled '*Polyporus nigricans*'. I think the correct name should have been *Poria obliqua*. Further evidence concerning the distribution of *P. obliqua* in Germany and Poland is, of course, necessary.

I wish to thank very much Mr E. G. Wyatt, the manager of Y.M.C.A. Hostel, Hallburn near Longtown, for the photograph of *P. obliqua*.

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EXPLANATION OF PLATE 8

Poria obliqua on *Betula pubescens* at Hallburn

(*Accepted for publication* 2 *May* 1949)

ECHIDNODES AULOGRAPHOIDES (BOMM., ROUSS. & SACC.) ROBERTSON COMB.NOV., IN BRITAIN

By NOEL ROBERTSON, *Botany School, University of Cambridge*

(With 1 Text-figure)

In the spring of 1944 and again in 1945 fructifications of a fungus which was clearly a member of the Microthyriales (sensu C. G. Hansford) were collected on the bark of living twigs of rhododendrons of the *Ponticum* series, at Dawick, Peebleshire, Scotland. The fungus agrees closely with the original description of *Lembosia aulographoides* Bomm., Rouss. & Sacc. (Saccardo, 1891), but it also agrees closely with the original description of *Microthyrium rhododendri* (Grove, 1933). Through the courtesy of Dr W. Robyns I have been able to examine specimens in the Herbarium of the Brussels Botanic Garden from the Herbarium of E. Bommer and E. Rousseau. Two of the packets are designated 'Typus' by the Belgian authorities and are labelled 'Tervueren, Juillet 1889, leg. M.R., E.B.'. It seems certain that these are in fact the type specimens on which Bommer, Rousseau and Saccardo erected *Lembosia aulographoides*. From the Brussels Herbarium I have also been able to examine Rehm's exsiccatus 1067 collected by Madame Destrée. Dr C. J. Hickman has kindly looked in Grove's Herbarium at Birmingham but can find no specimen of or reference to *Microthyrium rhododendri* Grove. There is, however, at Kew a specimen of *M. rhododendri* Grove. This specimen is from the herbarium of the Rev. P. G. M. Rhodes but is labelled entirely in Grove's handwriting. Moreover, after the name is written *nom. nov. ad interim*, and the locality and date are given as Caerdeon, Mer, 19. iv. 33. The original description cites a specimen collected in March 1933 from Caerdeon, and it is probable that the Kew specimen is a later collection from the type locality, but from the annotation it appears that this specimen was considered in the erection of the new species and it is probably a paratype.

The measurements and characters of these specimens are given in Table 1. No ascospores or asci could be found in Grove's specimen of *M. rhododendri* and his original description is inserted at the appropriate place.

Table 1

Character	My specimen	Grove's specimen	Bommer and Rousseau type of *Lembosia aulographoides*
External mycelium	Present	Present	Present
Hyphopodia	None	None	None
Thyriothecia	200–345 × 148–228 μ	135–351 × 108–202 μ	270–480 × 135–270 μ
Asci	24–44 × 21–32 μ	35–40 × 20–25 μ	34–44 × 24–31 μ
Ascospores (10 spores)	17–23 × 7–12 μ	20–24 × 9–10 μ	17–21 × 6–10 μ

There is no discrepancy between any of the measurements and the published dimensions. There is also a very close agreement between the

fungi examined, and there is no doubt that they fall within the same species. The presence of the external mycelium and the shape of the thyriothecium excludes the fungus from *Microthyrium*. On microscopic examination by reflected light it is generally not possible to observe any mycelium associated with the thyriothecia. Theissen (1913) was unable to demonstrate any mycelium when he examined Rehm's exsiccatus no. 1067 of *Lembosia aulographoides*, and for this reason he placed it in his new genus *Lembosina* of which it was the type. I have examined the type specimen of *Lembosia aulographoides* and Rehm's exsiccatus no. 1067 and in both, as well as in Grove's *Microthyrium rhododendri* and the fungus collected by me, have been able to demonstrate a well-developed ground mycelium, using the cellulose acetate stripping method.

According to Theissen & Sydow (1917) the fungus would fall in the genera *Lembosia* or *Echidnodes* according to the presence or absence of hyphopodia. There are certainly no lateral hyphopodia present, and the occasional swellings on the hyphae are not intercalary hyphopodia (*Cirsosia transversalis*, Herb. I.M.I. 13522, which has true intercalary hyphopodia, examined for comparison). Moreover, Mr M. B. Ellis of the Commonwealth Mycological Institute demonstrated the presence of a very fine internal mycelium under the cuticle and in the epidermal cells (a feature of *Echidnodes*) in one of my collections (R. 18, Herb. I.M.I. 34762). Theissen & Sydow (1917) and Stevens & Ryan (1939) make two genera *Echidnodes* and *Echidnodella* separated on the character of the presence or absence of paraphyses. Hansford (1946) maintains that the presence or absence of paraphyses is not a reliable character. In my own collections paraphyses are certainly present in young thyriothecia, but in older thyriothecia the substance surrounding the asci is a structureless mucus. Arnaud (1918) makes two genera *Balansina* and *Maurodothella* which approach close to *Echidnodes* and which Stevens & Ryan treat as synonymous genera. I have not had an opportunity of examining the type species of these genera and cannot say how the species under discussion relates to them. It certainly agrees with Theissen & Sydow's original definition of *Echidnodes*.

My collection is identical with the type specimen of *Lembosia aulographoides* Bomm., Rouss. & Sacc. and with the specimen of *Microthyrium rhododendri* Grove in the Herbarium, Kew, and all three are correctly referred to the genus *Echidnodes* of Theissen & Sydow (1917) as follows:

Echidnodes aulographiodes (Bomm., Rouss. & Sacc.) comb.nov.

Syn. *Lembosia aulographoides* Bomm., Rouss. & Sacc., Saccardo, *Sylloge fungorum*, **9**, 1107 (1891) and *Hedwigia*, **31**, 304 (1892).
Lembosina aulographoides (Bomm., Rouss. & Sacc.), Theissen, *Ann. mycol., Berl.*, **11**, 437 (1913).

Type material: 'In ramulis tenellis emortuis *Rhododendri pontici*, Tervueren Belgii.'

This species was treated as *Microthyrium rhododendri* by Grove (J. Bot., Lond., **71**, 287 (1933)). He erected a type for *M. rhododendri* on British material of his own collecting.

Type material: 'In ramulis emortuis v. semimortuis vetustissimorum *Rhododendrorum* cultorum, Caerdeon prope Barmouth, Mer., Mart., 1933.'

The fungus found by me occurs on the living twigs of rhododendrons of the *Ponticum* series. In these species the cortex is not cut off as bark until the second or third year. It was on such newly formed bark in the early spring that the ripe fructifications were found. In the areas on which the fungus occurred the bark took on a grey colour, distinct from the reddish colour of normal bark. The collections of Grove, Destrée, and Bommer and Rousseau were on dead twigs of rhododendrons. In the herbarium specimens some of the twigs are of considerable age and on these the fructifications and external mycelium are poor and the latter difficult to demonstrate. The best specimens containing asci and ascospores are found on the young twigs. I do not consider that the fungus I collected was either saprophytic or seriously pathogenic. In one instance fructifications of the fungus were found on a dead area of a leaf of rhododendron. Although Chinese rhododendrons were common in the same area none was infected with this fungus.

The fructifications occur on an external brown mycelium composed of smooth-walled anastomosing hyphae 2–3(4)μ in diameter. They are thyriothecia (see fig. 1), black, dimidiate and elongated 200–345 × 148–

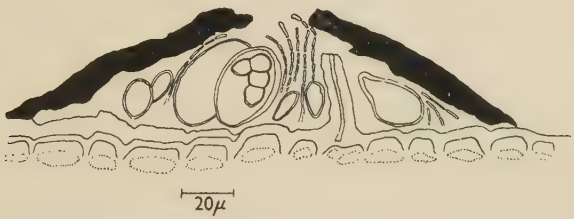

Fig. 1. *Echidnodes aulographoides*, thyriothecium in transverse section.

228μ; the cover is made up of radiating hyphae, it is dark and opaque in the centre becoming translucent towards the edges. The thyriothecia open by an irregular longitudinal slit which occasionally bifurcates. The asci, which are arranged in one basal layer, are globose and slightly thickened at the apex, 24–44 × 21–32μ, containing eight ascospores which are bicellular, constricted at the septum, with the lower cell slightly narrower than the upper one, and measuring 17–23 × 7–12μ. The ascospores are hyaline at first, becoming pale brown with verruculose walls. The hyaline spores are much the more numerous in all the preparations examined but the brown spores also belong to the fungus, for in a few preparations they have been found within an ascus. The paraphyses are demonstrable in a few preparations.

Collections examined

Herb. R.B.G. Kew; Brit. Col.: '*Microthyrium rhododendri*; nov.nom. ad interim, on branches of Rhododendron, Caerdeon, Mer. 19. iv. 33.' (scripsit Grove). Herbarium of the Rev. P. G. M. Rhodes, Beq. March 1935.

Herb. Hort. botan. Brux: '*Lembosia aulographoides* Bomm., Rouss. & Sacc. Sur de jeunes rameaux morts de *Rhododendron ponticum*. Parc de (? Verom), Tervueren, Juillet 1889. Leg. M.R., E.B.' Ex Herb. E. Bommer and M. Rousseau.

Herb. Hort. botan. Brux. Rehm's Ascomycetes no. 1067. '*Lembosia aulographoides* Bomm., Rouss. & Sacc. On *Rhododendron ponticum* aesten. Jardin aux environs de la Haye (Nederland), 1891.' Madame Destrée.

On Rhododendron twigs: Dawick, Peebleshire, Scotland, 19. ii. 44, leg. Noel Robertson, no. 17.

On Rhododendron twigs: Dawick, Peebleshire, Scotland, iv. 1945, leg. Noel Robertson, no. 18. (I.M.I. 34762.)

The occurrence of this member of the *Microthyriales* in Britain is of interest in itself, but it is also of practical importance to those who teach systematic mycology and who may not have access to herbarium material of these, usually tropical, fungi.

I am indebted to Dr W. Robyns, Director of the Brussels Botanic Garden, The Director, the Royal Botanic Garden, Kew, and Dr S. P. Wiltshire, Director, Commonwealth Mycological Institute, for facilities for the examination of specimens; to Miss E. M. Wakefield, Dr Malcolm Wilson and Messrs E. W. Mason, S. H. Hughes and M. B. Ellis for helpful discussion.

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FUNGI ASSOCIATED WITH AMBROSIA BEETLES IN GREAT BRITAIN

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(With Plates 9–11 and 2 Text-figures)

The symbiotic relationship of the ambrosia beetles and their associated fungi has been recognized for a long time. The beetles have been accurately described by many workers, but little attention seems to have been paid to the associated fungi. Leach, Hodson, Chilton and Christensen (1940) and Verrall (1943) in America, Webb (1945) in Australia, and Gadd and Loos (1947) in Ceylon, among others, have described these fungi, but no description of them has appeared in Britain. Ambrosia fungi serve as food for the beetles, and different fungi are cultivated by different species or groups of species. Webb (1945), however, thinks that all the ambrosia fungi from different parts of the world belong to the genus *Leptographium* and many may probably be referred to *L. lundbergii* Lagerberg and Melin. In view of this, the author studied the fungi associated with the ambrosia beetles in Great Britain to see how far they agree with those described by other workers.

Fungi associated with the beetles in host

Materials. Materials were collected from Blair Atholl and from the Crieff and Calley woods near Dunkeld, both being in Perthshire, Scotland. At Blair Atholl, standing trees of Japanese larch were dying over a wide area in the forest and their tops were killed. These trees were heavily infested by the ambrosia beetle, *Trypodendron lineatum*. The attack was confined to the lower half of the trees, being heaviest near the base and gradually diminishing towards the top. The trees were also infested by the wood-boring beetle, *Hylecoetus dermestoides*, and the bark-beetles, *Hylurgops palliatus* and *Dryocoetes autographus*. The healthy trees adjoining the infected ones had green tops and were free from beetle infestations. Material collected in September 1946 revealed the presence of typical *Trypodendron* galleries in the wood but neither beetles nor larvae were found. A search for the beetles in May 1947 was also unsuccessful, but late in June the same year, they were seen making fresh tunnels into the wood. It seems, therefore, that the beetles leave the trees in autumn and infest them again in late spring. It is not known how they survive the winter, but all the available evidence indicates that they do not overwinter in the trees.

In the Crieff and Calley woods felled logs of Japanese larch and Norway spruce that lay on the forest floors were heavily infested by *T. lineatum*. Early in July 1947 the beetles were seen just boring into the wood. The logs of Japanese larch were also infested by the bark-beetles, *Hylurgops palliatus* and *Dryocoetes autographus*, but those of Norway spruce were free from any

other beetle infestation. In the same forest, another species of *Trypodendron*, *T. domesticum*, was found infesting felled logs of birch, and larvae and adult stages were found in the tunnels. Isolations of fungi were always made from the farthest end of the tunnels, either from the fungus growing in them or by transferring to agar aseptically small slivers of wood from around the tunnels. Adult beetles were also allowed to walk on agar, when spores adhering to the body of the beetles were transferred to the medium.

Appearance of beetle tunnels. There is no marked difference between the patterns of tunnels bored by the two beetles, *T. lineatum* (Pl. 9, figs. 4, 6) and *T. domesticum* (Pl. 9, fig. 5). In general, the beetles attack only felled logs or dying trees, and the insects are never found in limbs where the sap flow is normal. The entrance holes are slender tunnels, slightly over 1 mm. in diameter and they penetrate into the sapwood (rarely into the heart wood) up to varying distances. Secondary galleries branch out from the entrance tunnel, being usually constructed along annual rings (Pl. 9, fig. 1). After copulation between the male and female beetles, the females begin to make egg-niches along the sides of the secondary galleries, vertically up and down, parallel to the grain of the wood (Pl. 9, figs. 2, 3 and 10). The tunnels in their earlier stages are colourless to light brown (Pl. 9, fig. 8). They soon develop a stain and become black. In conifers infested by *T. lineatum*, the stain diffuses into the sapwood up to 2–3 cm., the colour gradually fading away from the main tunnel (Pl. 9, figs. 2, 3). In birch infested by *T. domesticum*, the black stain occurs only round the tunnels (Pl. 9, fig. 10).

Appearance of fungi in the beetle tunnels. In the Japanese larch and Norway spruce collected both at Blair Atholl and Dunkeld, *Ceratocystis** *piceae* (Münch) Bakshi and *Leptographium lundbergii* were constantly isolated from the tunnels. Pl. 9, fig. 7, shows young conidiophores of *Ceratocystis piceae* round the entrance hole, while Pl. 9, fig. 8, shows coremia of the same fungus at the farthest end of a tunnel. This gallery had recently been made by the beetle, as is evident from the tunnel which is light coloured. When young, the conidiophore of *C. piceae* has a white and transparent stalk consisting of a single hypha with a colourless head (Pl. 9, fig. 7). From this simple conidiophore, all stages could be traced to the compound coremium of the species, where the stalk consists of agglutinated hyphae. The base of the stalk turns brown to black with age, the colour gradually fading towards the top, and the ultimate branches are colourless. Conidia borne at the ends of conidiophores are held in a drop of sticky mucilage, transparent at first, later becoming milk-white. In addition to the coremia, mycelium was found inside the tunnels, but this was scanty due probably to the beetles closely cropping it. The mycelia bore conidia which, when transferred to agar, gave rise to either *C. piceae* or *Leptographium lundbergii* or both. Perithecia with broken necks were occasionally seen in the previous years' galleries. The measurements of the perithecial bases were within the range of those of *Ceratocystis piceae*, but the bases were empty and contained no spores. The fungi isolated from the gallery were not contaminations introduced into the tunnels by the predator beetles which usually follow

* The nomenclature of *Ceratostomella* Sacc., *Ceratocystis* Ellis & Halst. and other allied genera is dealt with elsewhere (Bakshi, 1950).

the *Trypodendron* beetles into the wood. The fungi were frequently isolated from beetle galleries which had not been invaded by predators and the spruce logs were infested only by *T. lineatum.*

It was only from the larch collected at Dunkeld that a third fungus, *Oedocephalum lineatum* n.sp., was isolated. This fungus grew pure or intermixed with the coremia of *Ceratocystis piceae* in the gallery (Pl. 9, fig. 9). It produced white mycelium, with closely septate, thin-walled, branched hyphae having colourless granular contents, 2·6–3·5 μ, average 3·0 μ broad. Conidiophores were produced abundantly; they were colourless, usually non-septate (Text-fig. 1 *a*, *b*) and up to 175 μ long. The apices were swollen into a head and carried sterigmata, on each of which a single conidium was borne. The conidia were pear-shaped (Text-fig. 1 *e*) with their pointed ends attached to the sterigmata and were borne in groups of 15–20 at the head. They were colourless, 1-celled and measured 5·1–7·0 × 2·5–4·1 μ, average 5·6 × 3·2 μ.

No obvious fungus growth was found in the galleries of *Trypodendron domesticum* infesting birch. Cultures started by transferring slivers of wood from the tunnels yielded a new species of *Ceratocystis, C. ambrosia.*

Life history of ambrosia fungi in culture

The cultural characters of the fungi were studied on malt agar in Petri dishes kept in darkness in incubators at 22° C. and also on wood. All measurements represent an average of thirty counts.

Ceratocystis piceae (Münch) Bakshi n.comb.

Syn. *Ceratostomella piceae* Münch
Ophiostoma piceae (Münch) H. & P. Syd.

The development of *C. piceae* in culture has been described by Münch (1907), MacCallum (1922) and Lagerberg, Lundberg and Melin (1928), and the observations given below will be limited to some features not mentioned by previous workers.

The fungus grows readily on agar (Pl. 10, fig. 12) and on sterile pieces of spruce (Pl. 10, fig. 14), pine and larch. Aerial mycelium bears conidia of the *Cladosporium* and *Cephalosporium* types (Pl. 10, fig. 13). Coremia develop abundantly and may bear daughter coremia. When this happens, the hyphae from the stalk of the mother coremium elongate through the head and form the stalk of the daughter coremium (Pl. 9, fig. 11). The secondary coremia are thus genetically connected with the mother and not, as Lagerberg *et al.* (1928) thought, epiphytes independent of the parent.

Perithecia develop abundantly in culture, both on agar and on wood (Pl. 10, fig. 14). Münch (1907) and Lagerberg *et al.* (1928) found difficulty in obtaining perithecia in culture and sometimes they were absent: Lagerberg *et al.* produced a few on a very acid medium.

The fungus was also isolated from felled logs of Japanese larch in the forest at Glentress, Peebles, Scotland. Coremia and perithecia developed underneath the bark. Several specimens of blued pine boards collected near

Gatehouse of Fleet, Kirkcudbrightshire, Scotland, yielded *Ceratocystis piceae* and *C. coerulescens* (Münch) Bakshi (syn. *Endoconidiophora coerulescens* Münch) in culture when pieces of blued wood were transferred to agar. No fruiting structures developed on these pine boards.

Leptographium lundbergii Lagerberg and Melin

L. lundbergii is the type species of the genus *Leptographium* created and described by Lagerberg *et al.* (1928) from Sweden. It has been recorded from Australia and the States and thus appears to have a wide geographical distribution. In Britain, the fungus was probably observed by MacCallum (1922), as pointed by Lagerberg *et al.* (1928), but its independent position was not ascertained and the present record is thus the first authentic one for Britain.

The fungus is fast growing, radial growth being 8 cm. in ten days. Mycelium is sparse on the medium (Pl. 10, fig. 15). Earlier shades of colour consist of Dusky Olive Green (Ridgway, 1912) and Lincoln Green, which with age deepens to Blackish Mouse Gray with patches of brown shades consisting of Fuscous and Clove Brown.

In young cultures conidia are borne singly or in heads, but soon typical conidiophores of the *Leptographium* type appear (Pl. 10, fig. 16). The conidia are held at the top of the conidiophores in a milky drop which assumes a yellow or brown colour with age: they are thick-walled with greenish granular contents, and measure $6{\cdot}7–12{\cdot}7 \times 3{\cdot}3–6{\cdot}0\,\mu$, average $9{\cdot}5 \times 4{\cdot}2\,\mu$. The hyphae are thin-walled when young but become thick-walled and brown with age. They are usually $5–6\,\mu$ broad but may be up to $13\,\mu$ thick.

The fungus grows readily on pine, spruce and larch, and imparts to all wood an intense blue colour within a short time. The hyphae penetrate the wood readily and are abundant in the pith rays (Pl. 10, fig. 17) as black lines filled with thick-walled, dark brown hyphae. They are also present in tracheids. Penetration between adjoining tracheids occurs frequently through bordered pits.

The fungus was also isolated from intensely blued pine boards in a saw mill in Edinburgh.

Oedocephalum lineatum n.sp.

On agar. The fungus grows readily, producing a white mycelium (Pl. 11, fig. 18). Aerial mycelium is at first scanty but soon numerous conidiophores appear and this gives the mat a powdery appearance, chalk-white in colour. The rate of radial growth is about 5 cm. in ten days. In a month-old culture in tubes, the aerial mycelium is abundant, giving a felty appearance to the mat. Pigmentation develops in small areas only or may be uniformly present all over the mat. This consists of shades of Vinaceous Fawn, Light Vinaceous Cinnamon, Light Pinkish Cinnamon, Cinnamon, Light Congo Pink, Vinaceous Pink and Light Cinnamon Drab. Pigmentation in Petri dish cultures is not so varied and consists of shades of Light Pinkish Cinnamon.

Conidia are borne only on slender conidiophores which arise vertically

from the substratum hyphae (Pl. 11, fig. 19). The conidiophores average 180 μ but may be up to 220 μ long. They may be non-septate (Text-fig. 1 *c*) or septate (Text-fig. 1 *d*) by transverse walls, thick-walled with hyaline

Text-fig. 1. *Oedocephalum lineatum*. *a*, conidiophores from gallery of *Trypodendron lineatum* with conidia at the head; *b*, same, but conidia removed to show sterigmata at the head; *c*, conidiophores from culture; *d*, same showing septate nature; *e*, conidia from nature; *f*, conidia from culture. *a*, *b*, *d*, *e*, *f*, ×960; *c*, ×570.

granular contents, 6–7 μ broad swelling at the head to 15 μ across. On the swollen head, numerous short, slender, pointed sterigmata (Text-fig. 1 *c*, *d*) are borne and each bears a conidium. The conidia are pear-shaped or round with pointed ends (Text-fig. 1 *f*) by which they are attached to the

sterigmata. They are thick-walled, 1-celled with colourless, granular contents, and measure 4·3–6·6 × 3·0–5·3 μ, average 5·47 × 4·25 μ.

Young hyphae are colourless, thin-walled with hyaline granular contents, 2–3 μ broad but may be up to 6 μ in diameter: with age, they become light brown.

On wood. The fungus develops readily on all the kinds of wood used for *Ceratocystis piceae* and produces a white mycelium and abundant conidiophores.

The fungus agrees closely with *Oedocephalum preusii* Sacc. (Massee, 1893), but differs in having conidiophores without bulbous bases and in much smaller conidial measurements.

Latin diagnosis. Myceliis juvenilibus hyalinis maturis brunnescentibus puniceis; hyphis juvenilibus tenui-tunicatis hyalinis scenescentibus brunneis 2–3–6 μ latis; conidiophoris copiosis septatis vel aseptatis haud ramosis crasso-tunicatis 220 μ longis, 6–7 μ latis, sed capitibus ipsis 15 μ latis; conidiis (e sterigmatibus nascentibus) piriformis crasso-tunicatis hyalinis 4·3–6·6 × 3·0–5·3 μ.

In cuniculis *Trypodendri lineati* in truncis *Laricis leptolepis* Dunkeld, Perthshire, Scotland.

Ceratocystis ambrosia n.sp.

On agar. The growth is appressed and sodden with very scanty aerial mycelium (Pl. 11, fig. 20). The rate of radial growth is about 3·5 cm. in ten days. Mycelium is mostly colourless in Petri dishes, while in tubes pigmentation appears and consists of shades of Deep Mouse Grey to Dark Mouse Grey.

Conidia begin to appear in about a week and are borne in groups on short aerial hyphae arising from the substratum or on superficial mycelium (Text-fig. 2*b*). They are 1-celled, hyaline, elongate (Text-fig. 2*c*) and measure 4·7–14 × 2·7–5·7 μ, average 7·8 × 3·8 μ.

Perithecia begin to appear in cultures about two weeks old and they mature after about three weeks. The base of the young perithecium is brown and pseudoparenchymatous but becomes black on maturity (Pl. 11, fig. 24). The ventral diameter and height of the perithecial base vary from 109 to 221 μ and 103 to 214 μ (average 165 and 157 μ), respectively. The base of the perithecium is covered with ventral hairs, 12–50 μ long, 3–3·3 μ broad at the base and 2·3–3 μ at the tip. They are thick-walled, unbranched, brown at the base and colourless at the tip. The length of the neck varies from 240 to 924 μ, including cilia when present, average being 490 μ. The neck is 32–49 μ, average 37·5 μ broad at the base, 9·8–15·4 μ, average 12·6 μ at the tip. The neck is black except at the tip where the colour fades to light-brown. Cilia may be absent at the ostiole or may be present (Text-fig. 2*d*), the maximum count made being ten. They vary in length from 21 to 25 μ, though occasionally cilia 10 μ long may be seen, and are mostly 1-celled, rarely 2–3-celled by cross-walls, thin-walled, hyaline, 1·7–2·3 μ broad at the base, tapering to 0·8–1·2 μ at the tip.

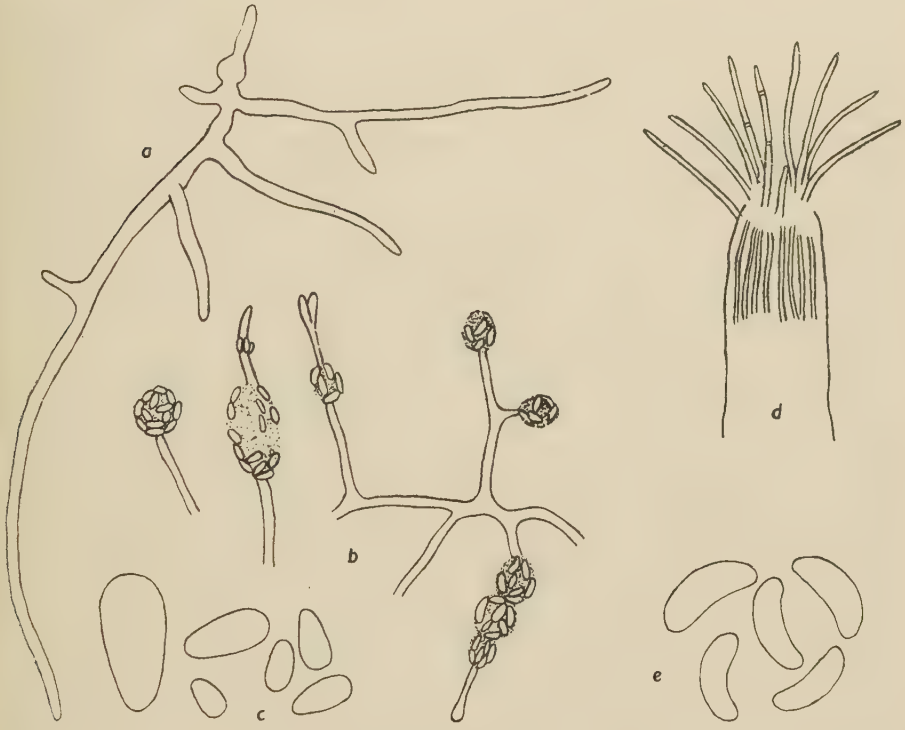
When mature, the ascospores collect at the ostiole embedded in mucilage, milk-white at first and turning yellow with age. The spores are hyaline, 1-celled, thin-walled, kidney-shaped (Text-fig. 2*e*) and measure 3·8–5·3 ×

1·3–2 μ, average 4·5 × 1·66 μ. They germinate by many germ-tubes (Text-fig. 2*a*).

Sclerotia are produced abundantly in culture. They are round, dark-brown to black bodies and measure 50–65 μ in diameter (Pl. 11, fig. 21).

Hyphae when young are thin-walled and hyaline with hyaline, non-granular contents, but with age they become thick-walled and dark-brown in areas where coloration develops. Breadth varies from 2 to 5 μ.

The fungus is heterothallic. Monoascospore cultures produce conidia and sclerotia only and perithecia are formed only when two mycelia of



Text-fig. 2. *Ceratocystis ambrosia*. *a*, germinating ascospore; *b*, conidia in heads on aerial hyphae; *c*, conidia; *d*, top of perithecial neck showing crown of cilia; *e*, ascospores. *a*, *b*, × 570; *c*, *e*, × 3100; *d*, × 760.

opposite strains meet (Pl. 11, figs. 22, 23). Numerous pairings between eight monoascospore mycelia revealed the presence of only two strains in the fungus.

The fungus agrees closely with *Ceratostomella shrenkianum* Hedgcock (1906) but differs in the absence of the dendroid fruiting tuft and in conidial, perithecial and ascospore measurements.

Latin diagnosis. Myceliis appressis, juvenilibus hyalinis, maturis brunnescentibus nigris; hyphis juvenilibus hyalinis tenui-tunicatis, maturis brunnescentibus tandem crasso-tunicatis 2–5 μ latis; conidiis ad formam in genere *Cephalosporio*; unicellulis hyaliniis elongatis 4·7–14·0 × 2·7–5·7 μ;

peritheciis copiosi juvenilibus basi brunnescentibus pseudoparenchymatis, senescentibus nigris hirsutis, perithecii ventre 109–221 μ lato, 103–214 μ alto; rostello 240–924 μ longo, 9·8–49·0 μ lato, ostioli filamentis obsoletis vel 1–10 hyalinis tenui-tunicatis 10·0–25·0 μ longis, 0·8–2·3 μ latis; ascosporis hyalinis unicellulis reniformis 3·8–5·3 × 1·3–2·0 μ; sclerotiis brunnescentibus aut nigris ± globosis 50–65 μ latis.

Fungus heterothallicus. In cuniculis *Trypodendri domestici* in truncis *Betulae albae*. Dunkeld, Perthshire, Scotland.

Discussion

Ambrosia fungi described by different workers have been reviewed by Leach *et al.* (1940) and Webb (1945). Webb, who worked with Australian ambrosia fungi, isolated *Leptographium lundbergii* and two species of yeasts belonging to *Endomycopsis* from the tunnels of *Platypus subgranosus*. She tried to point out the resemblance between the ambrosia fungi described by previous workers and *Leptographium* and concluded that all ambrosia fungi from different parts of the world belong to that genus and could probably in many cases be referred to *L. lundbergii*. My observations, however, lead me to differ from this view. Four species of fungi, including *L. lundbergii*, were isolated pure from the galleries of the two ambrosia beetles and it is my opinion that ambrosia beetles cultivate different but specific fungi in their galleries. Neger (1909, 1924) stated that *Endomyces* sp. and *Graphium* sp., the latter being extra fructifications of *Ceratostomella*, were found in the galleries of *Xyleborus lineatus*. *Graphium* sp. was not identified and it is quite probable that Neger observed a species closely related to, if not, *Ceratocystis piceae*. Doane and Gilliland (1929) found a species of *Ceratostomella* in association with *Gnathotrichus sulcatus*. Two distinct forms, conidial and perithecial, were observed in the tunnels. The fungus was not identified though it appears that the authors were dealing with species of *Ceratocystis*. Two species of *Ceratocystis* were isolated from the beetle tunnels of *Trypodendron lineatum* and *T. domesticum*, and it seems that the association between *Ceratocystis* and the ambrosia beetles, as with bark-beetles, is much more significant than has previously been supposed. The species of *Ceratocystis* are suitably adapted to being disseminated by the beetles. Spores are embedded in sticky mucilage at the top of the perithecial neck, and conidia are similarly embedded at the heads of coremia when present, and these are therefore conveniently carried away on the body of the beetles. The mucilage also prevents complete digestion of spores in the alimentary canal of the beetles and it is likely that some of them remain in a viable condition after they have passed out of the body of insects and lain in the insect tunnels along with faeces.

It is possible for the ambrosia fungi to occur in nature unassociated with beetles or in association with bark-beetles. *C. piceae* was isolated on several occasions from the bark of felled logs of larch. *Leptographium lundbergii* was isolated from a blued pine board and was similarly isolated by Lagerberg *et al.* (1928). Rumbold (1931) isolated the fungus from pine infested with the bark-beetle, *Dendroctonus monticolae*. The bark of pine from which MacCallum (1922) isolated *Ceratocystis piceae* and which was probably

contaminated by *Leptographium lundbergii* was riddled with holes made by the bark-beetle, *Hylesinus piniperda*.

A true case of symbiotic relationship exists between the ambrosia beetles and their associated fungi. The beetles help the fungi in their dissemination and thus open up new and suitable substrata for them; while the fungi in their turn serve as food for the beetles. I did not carry out feeding experiments on the beetles to see whether the larvae could be brought from egg to pupation on cultures of their respective fungi. Sections of beetles and their larvae showed the presence of spores in their alimentary canals. As the fungi were constantly isolated from the galleries of ambrosia beetles from different localities under pure condition, I believe that they should be considered as 'ambrosia' in the true sense.

Summary

The fungi associated with the ambrosia beetles infesting dead and standing trees and logs in Britain have been isolated. *Ceratocystis piceae* (Münch) Bakshi, *Leptographium lundbergii* Lagerberg and Melin and *Oedocephalum lineatum* n.sp. were constantly isolated from the gallery of *Trypodendron lineatum* infesting conifers in different localities in Scotland. *Ceratocystis ambrosia* n.sp. was isolated from the tunnels of *Trypodendron domesticum* infesting birch.

The behaviour of the ambrosia beetles inside the wood has been studied and the appearance of the beetle tunnels and the fungi growing in them are described both on agar and on various species of coniferous wood.

Leptographium lundbergii is recorded in Britain for the first time. It has been found to be a strong blueing agent on various coniferous timber, whereas *Ceratocystis piceae* has a weak capacity for blueing wood.

The ambrosia beetles are believed to cultivate different but specific fungi in their galleries. The symbiotic relation between the ambrosia beetles and their associated fungi is discussed. The beetles help the fungi in their dissemination and thus open up new and suitable substrata for them. The fungi, in their turn, serve as food for the beetles. Though no feeding experiments were performed, it is assumed that the fungi, isolated from the beetle galleries, are 'ambrosia' in the true sense since they were isolated constantly from the beetle tunnels under pure conditions from different localities.

The author wishes to thank Dr Malcolm Wilson who suggested this problem and under whose supervision it was carried out. He is indebted to Prof. Sir W. W. Smith for the Latin diagnosis of the new species and to Mr E. W. Mason for advice on the nomenclature of *Ceratocystis*. Cultures of all the species described have been deposited at the Commonwealth Mycological Institute, Kew, at the Forest Products Research Laboratory, Princes Risborough and in the Botany Department of Edinburgh University.

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EXPLANATION OF PLATES 9–11

Fig. 1. Transverse section of the trunk of Japanese larch showing entrance hole and lateral galleries along annual rings constructed by *Trypodendron lineatum*. × reduced.
Fig. 2. Longitudinal section of Japanese larch showing tunnels in the wood. Note the dark stain in the tunnels. × reduced.
Fig. 3. Secondary gallery and larval chambers of *T. lineatum*. × 1½.
Fig. 4. *T. lineatum*. × 4.
Fig. 5. *T. domesticum*. × 4.
Fig. 6. *T. lineatum* inside the tunnel. × 2.
Fig. 7. Young conidiophores of *Ceratocystis piceae* at the entrance hole of *Trypodendron lineatum*. × 10.
Fig. 8. End of a gallery of *T. lineatum* on Norway spruce showing coremia (*c*) of *Ceratocystis piceae*. × 3.
Fig. 9. Conidiophores of *Oedocephalum lineatum* in the gallery of *Trypodendron lineatum* on larch. × 28.
Fig. 10. Longitudinal section of birch showing secondary galleries and larval chambers of *T. domesticum*. × 2.
Fig. 11. Coremium of *Ceratocystis piceae* formed in culture showing proliferation. × 67.
Fig. 12. Thirty-day-old culture of *C. piceae* on agar. × reduced.
Fig. 13. Aerial mycelium of *C. piceae* in culture showing strands bearing conidia. × 56.
Fig. 14. Artificial culture of *C. piceae* on Norway spruce. Note abundant perithecia and coremia. × 4.
Fig. 15. Ten-day-old culture of *Leptographium lundbergii* on agar. × reduced.
Fig. 16. Aerial mycelium of *L. lundbergii* in culture showing conidiophores of *Leptographium* type. × 8.
Fig. 17. Longitudinal radial section of Scots pine artificially inoculated with *L. lundbergii* showing hyphae in the rays. × 75.
Fig. 18. Ten-day-old culture of *Oedocephalum lineatum* on agar. × reduced.
Fig. 19. Aerial mycelium of *O. lineatum* in culture showing conidiophores. × 63.
Fig. 20. Thirty-day-old culture of *Ceratocystis ambrosia* on agar. × 1.
Fig. 21. Sclerotium of *C. ambrosia* from culture. × 220.
Fig. 22. Pairing of two monoascospores of different strains showing perithecia at their line of union. × reduced.
Fig. 23. Pairing of two monoascospores of different strains showing perithecia at their line of union. Magnified view of the line of union to show perithecia and sclerotia. × 4.
Fig. 24. Perithecium of *C. ambrosia* from culture. × 100.

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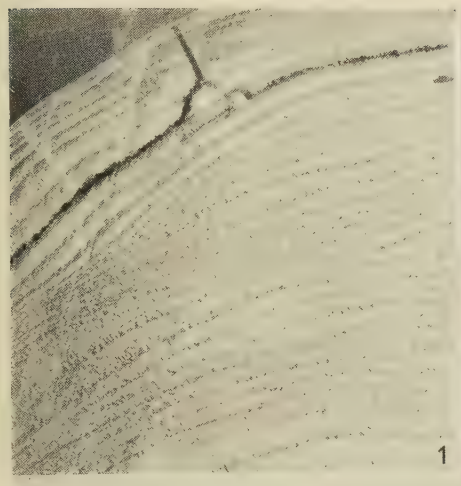
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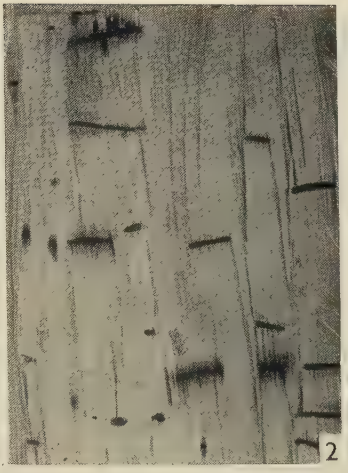
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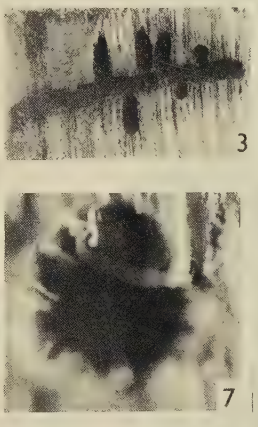
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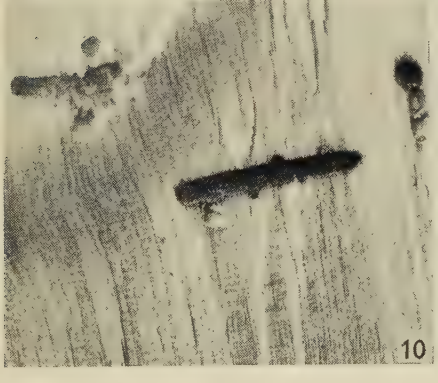
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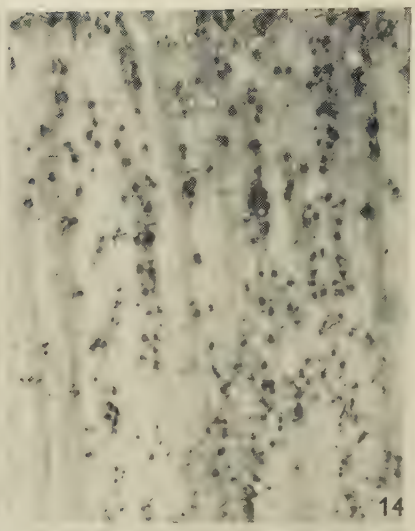
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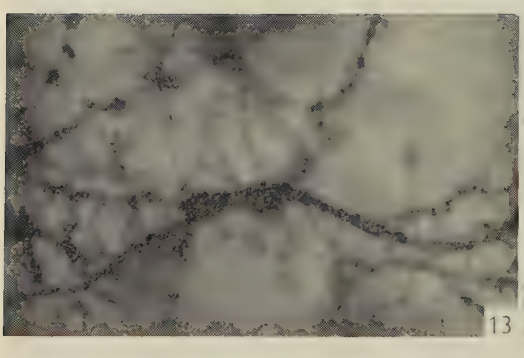
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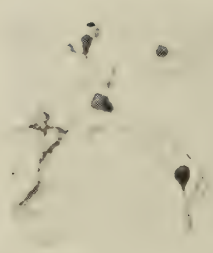
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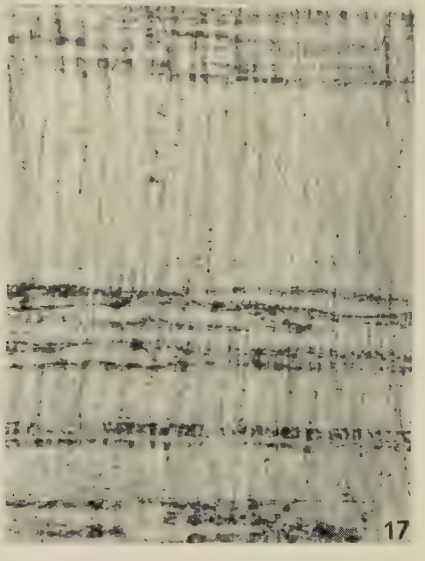
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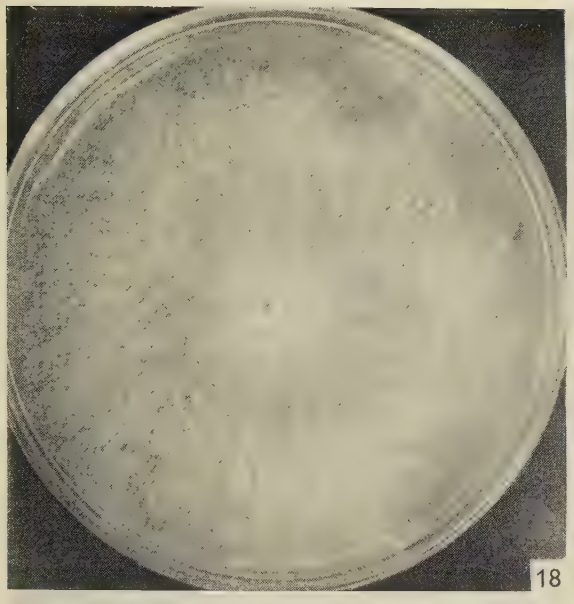
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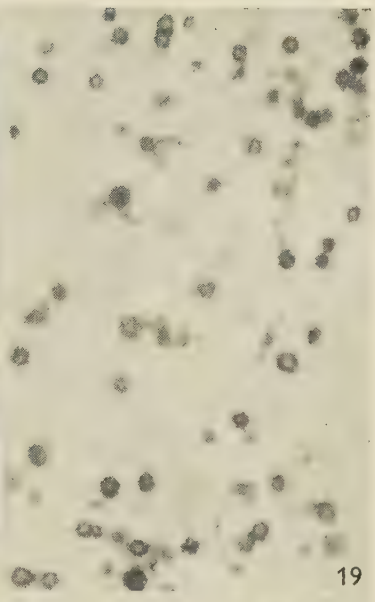
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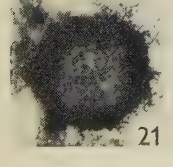
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OBSERVATIONS ON STRAINS OF *FUSARIUM OXYSPORUM* FROM THE OIL PALM AND BANANA FROM CENTRAL AND WEST AFRICA

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(With Plate 12 and 2 Text-figures)

I. Introduction

In 1946, Wardlaw (1946*a*, *b*), described a new vascular wilt disease of the oil palm (*Elaeis guineensis*) in the Belgian Congo, the strains of *Fusarium oxysporum* Schl. isolated being studied on standard media and described by Gogoi (1949). In 1947, the disease was described as occurring in Nigeria by Messrs S. de Blank and F. Ferguson (private report) and this finding was confirmed by Wardlaw (1948); in the latter report, mention is made of the fact that infected strands could be observed in seedling palms of eighteen to twenty-four months old, and strains of *Fusarium* isolated. Thirty-five cultures isolated during these initial investigations of wilt disease in Nigeria and the Belgian Congo have come into my hands, including two cultures of *F. oxysporum* from cases of banana wilt disease in the Cameroons. A further collection of four cultures, freshly isolated from the oil palm, were received during the course of the investigation and these also are considered here.

In the investigations of this important disease of the oil palm, the inoculation experiments will have to be carried out on the spot as soon as possible. Arrangements for this have been made. Meanwhile, my mycological investigations have aimed at contributing to our knowledge by providing information on the following aspects:

(*a*) The morphology of *F. oxysporum* and other species of *Fusarium* from the oil palm and banana on a standard synthetic medium, comparison being made with *strain W*—a wild strain isolated from necrosed vascular strands—already described by the writer (Gogoi, 1949).

(*b*) Evidence of mutation in the wild type.

(*c*) Growth of the strains in soil culture.

(*d*) The effect of synthetic as compared with natural potato-dextrose agar on the growth of the wild type and its mutants.

It is a matter of some interest and importance to know if the wild type of *F. oxysporum* isolated from the oil palm shows a high degree of morphological uniformity, how it compares with strains pathogenic to the banana, if it has a high rate of mutation, and how its mutant types may be distinguished from the parent wild type.

II. Materials

The materials available for study consisted of thirty-three isolations of *Fusarium* from diseased vascular strands of seedling and adult palms and

two from vascular wilt disease of bananas in the Cameroons. Of the thirty-three isolations from the palm, four proved to be *F. solani* and one *F. dimerum* and twenty-eight *F. oxysporum*. These isolations were obtained from West and Central Africa during a period of three years from 1946 to 1949.

III. Experiments and observations

Growth on the standard synthetic medium

The twenty-eight isolations of *F. oxysporum* from palms were grown on a standard synthetic potato-starch-dextrose agar* (Gogoi, 1949). This medium will be referred to as standard synthetic medium in this paper. The concentration of potato starch used in these cultures was 5%, as this concentration was found to be the best for colour reaction which is produced promptly after six to seven days. Plates were inoculated with conidial suspensions from young cultures (eight to ten days old) to limit the occurrence of mutants and incubated at 24° C. The main object of this series of experiments was to observe and compare the morphological characters and the colour reactions of the isolations in the standard synthetic medium. The data set out in Table 1 indicate the extent of resemblances and variations observed in the twenty-eight isolations grown from single conidia.

Table 1. *Characters shown by twenty-eight isolations of* Fusarium oxysporum *in standard synthetic medium*

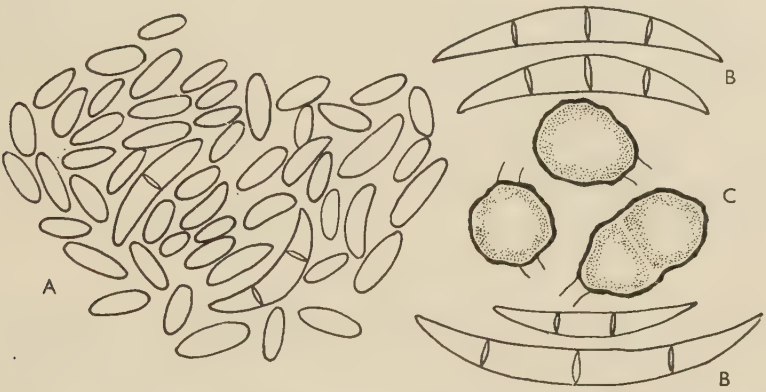
Pigmentation of medium after 2 weeks (Ridgway 1912)	'Indigo blue' colour in patches or in the form of rings near the centre. The colour differed in intensity and time required for development. A slight 'orange pink' coloration was associated with most of the cultures
Mycelium	Luxuriant aerial mycelium; highly septate with swollen hyphal cells in five cultures.
Sporulation	Dense, microconidia predominant Eight cultures showed 0·5–7·0% macroconidia Nine cultures showed approximately 40% macroconidia One culture showed macroconidia only Ten cultures showed microconidia only
Curvature of macroconidia	Slight curvature, usual; not curved, occasional
Attachment of conidia	Directly abstricted
Chlamydospores	Present in all cultures except one;[1] diameter 4·5–9 μ
Sclerotia	Present in eleven cultures
Sectoring	Present in four cultures

[1] This culture was isolated from a seedling palm in Nigeria. The macroconidia were abundant, long and highly septate, chlamydospores were absent.

Average conidial measurements were as follows, Text-fig. 1: 0-septate, $3{\cdot}0 \times 1{\cdot}5\,\mu$; 1- to 3-septate, $13{\cdot}5 \times 3\,\mu$ to $33{\cdot}0 \times 4{\cdot}5\,\mu$; chlamydospores, diameter 4·5–9·0 μ.

* *Composition of the medium*: glucose 2 g., potato starch 50 g., $NaNo_3$ 2 g., K_3PO_4 1·25 g., $MgSO_4$ 0·75 g., agar 15 g., water, 1000 ml.; pH adjusted to 5·6.

Broadly speaking, the twenty-eight isolations identified as *F. oxysporum* formed a close assemblage of strains. The accessions of 1947, 1948 and 1949 were, in fact, closely comparable with *strain W* as described by me (Gogoi, 1949). Some variation was, however, observed in respect of coloration, sporulation, production of sclerotia and sectoring. A mutant form which has been investigated in some detail, produces abundant, more or less straight and highly septate macroconidia, and has not the capacity to produce the 'indigo blue' colour on the standard synthetic medium. As a fact of experience in subculturing these strains, it has been found that there is a tendency to select the mutant. Hence, as the collection of isolations was obtained over a period of two years before this comprehensive investigation was begun, the several groups of strains as indicated by the formation of macroconidia (Table 1) may represent the progressive stages in replacement of the parent by the mutants. The patchy distribution of blue colour in the medium shown by a few cultures affords further evidence of this change.



Text-fig. 1. × 1000. A, a group of conidia of *F. oxysporum* wild strain from a culture on standard synthetic medium showing a characteristic assemblage; B, a group of typical 3-septate macroconidia of the wild strain; C, a group of chlamydospores of the same.

Growth of a few strains in synthetic and natural potato-dextrose agar

In that a considerable amount of systematic investigations of species of *Fusarium* has been undertaken on potato-dextrose agar (using potatoes), and since the writer had specified the characters of the oil palm wilt organism on a standard synthetic medium, it seemed desirable to make a comparison of strains grown on the two media. The investigations were, therefore, further extended by studying the effect of synthetic as compared with natural potato-dextrose agar on the growth of the wild type and its mutant and a few other selected strains.

The media used for this series of experiments were:

(1) Standard synthetic medium—with 5% potato starch.
(2) Standard synthetic medium—with 20% potato starch.
(3) Potato-dextrose-agar—20% potato decoction, 2% dextrose.

The data of this experiment showed that the characteristic blue coloration on the standard synthetic medium was not in any instance observed on the natural potato-dextrose medium. Moreover, the synthetic medium served to distinguish the mutants from the parental type, the former showing no colour development (Pl. 12).

Evidence of mutation in the wild type

During the course of the investigation, as mentioned above, a conspicuous new strain was accidentally obtained from a wild strain (strain W) which failed to produce the 'indigo blue' colour in the standard synthetic medium. This new strain differed from the parent strain in morphology and in some physiological characters. It has retained its distinguishing characters unaltered, and is regarded as being a mutant form. Attempts have been made without success to obtain other mutants by subculturing wild strains in a wide range of synthetic and natural media using either the hyphal tip method or by picking up single conidia. In some of these cultures sectors were not of infrequent occurrence, but such further cultural work as could be done indicated that these were only minor saltants not differing conspicuously from the parent culture (Pl. 12). Moreover, none of these saltants was comparable to the mutant except in the fact that a few of them did not develop the 'indigo blue' colour in the standard synthetic medium. No other mutants with different and constant characters were obtained during the course of investigations. These and other facts indicate that *F. oxysporum* as obtained from the vascular strands of the oil palm, shows considerable uniformity and stability in its cultural characters.

Pigment formation in the wild type

The production of pigment in the standard synthetic medium by the wild type is a point of interest. This strain always produced a strong 'indigo blue' pigment in this medium either in the form of a ring 2 cm. away from the centre or throughout the medium. The pigment was found diffused in the medium. When dilute sulphuric acid or hydrochloric acid was added to a plate the 'indigo blue' colour was immediately changed to pink, showing that the pigment behaves to some extent as an indicator. The aerial mycelium was normal, colourless and bearing abundant microconidia. Hyphae from the substratum were broad, swollen and highly septate, and looked purple under the microscope.

Some work has been done on the formation of pigment by *Fusarium* (Brown, 1925). In particular, the carbon:nitrogen ratio in the medium was found to affect it. By way of extending this observation a series of liquid and solid media was made up with different concentrations of potato starch, the amount of nitrogen being kept constant. The concentrations of potato starch used were 0·2, 0·5, 1·0, 2·5, 5, 10, 15 and 20%. The pH of all media was adjusted to 5·6. The wild and mutant strains were grown in these media and the cultures were examined after two weeks. Both strains showed better aerial mycelial growth in the higher concentrations of potato starch. The final pH of all cultures of the wild strain was found to be approximately 8 (determined by universal indicator), regardless

of the initial differences in the concentration of potato starch. The wild strain alone developed the colour and that only in the media with 2·5, 5, 10, 15 and 20% potato starch; i.e. no colour was developed in the low concentrations. This confirms the observations of earlier workers that high carbon : nitrogen ratio affects pigment formation in colour-producing media. These observers noted a slightly acid reaction in the medium, and thought that this might be a factor in the formation of pigment. But in the experiment described here, all the cultures showing colour developed a highly alkaline reaction. As the liquid cultures grew older, the colour became purplish and the reaction in the medium was observed to be slightly acid on a litmus test. The pigment in agar cultures was soluble in water and alcohol, and this was borne out by the liquid cultures. When slightly acid, red solutions were treated with an alkali they became blue. And again, when the wild strain was grown in acid media, the 'indigo blue' colour did not appear until the medium attained a pH of approximately 8. The actual colour, red or blue, is determined by the acidity or alkalinity in the medium. Whether there is a relationship between the presence of staling substances and pigment formation is not known. In other experiments it was found that the wild strain produced the indigo blue colour over a wide pH range (from 3·8 to 8·4), but was first evident in the medium with pH 8·4.

Maintenance of the wild characters in soil culture

It has been noted that the majority of the cultures obtained from West and Central Africa showed a predominance of microconidia; a few cultures showed microconidia only. But all of them produced the 'indigo blue' colour, either in patches or in the form of a ring after six to ten days' growth in standard synthetic medium. These two characters—the predominance of microconidia and the production of 'indigo blue' colour in the medium—have therefore been regarded as conspicuous diagnostic characters of the wild type. As *F. oxysporum* is a soil fungus, subject to mutation as described above, it was a matter of interest and practical importance to ascertain if the pathogen maintained its original wild characters in soil culture, or whether it tended to be replaced by mutant forms.

For these experiments, an isolation with microconidia only was selected. The fungus was grown in sterilized loam soil and subcultured from soil to soil, at intervals of two weeks. Loam soil in pots and tubes (slopes) was used. The pots were inoculated from the bottom with a conidial suspension in sterile distilled water. Controls for sterility tests were also set up. When a soil to soil transfer was made, the fungus was also subcultured in standard synthetic agar slopes to test for the production of the 'indigo blue' pigment and sporulation.

The records show that no change whatsoever had taken place up to the fifth soil subculture. In the sixth soil subculture, however, a change in sporulation occurred, some (about 1–2%) 1- to 3-septate, slightly curved macroconidia being present. The subculture from this culture, however, still produced the characteristic 'indigo blue' pigment. When conidial suspensions of this subculture were spread out in 3% malt agar and fifty

germinating single conidia (twenty-five macro- and twenty-five microconidia) were picked up and transplanted into slopes of standard synthetic medium, all developed the characteristic pigment after one week, and the sporulation was substantially the same as that observed in the soil culture. Moreover, these macroconidia were characteristic of the wild type. These observations suggest that the oil palm wilt *Fusarium* can persist in the soil for a considerable period of time, maintaining its wild characters. Whether the rate of mutation in the soil is lower than in synthetic culture media is a matter of interest and possibly of practical importance and deserves further investigation.

Further observations on freshly isolated strains from the oil palm

To confirm the characters attributed here to the wild strain of *F. oxysporum* a mycological worker in the Belgian Congo was asked to send fresh isolations. Accordingly, pieces of necrosed vascular tissue from wilted oil palms were placed directly on malt-agar slopes and dispatched to Manchester. Four such initial isolations were received in March 1949; these were seventeen days old on arrival. Observations made on these original cultures showed that all of them had moderate mycelial growth, a deep 'corinthian red' pigment and a predominance of microconidia. 500 conidia from each isolation counted in groups of 100 yielded the data set out in Table 2.

Table 2. *Percentage septation of conidia*

Isolation	Mean percentage septation	
No. 1	0-septate	98·2
	1-septate	1·0
	2-septate	0·4
	3-septate	0·4
No. 2	0-septate	95·0
	1-septate	4·0
	2-septate	0·6
	3-septate	Nil
No. 3	0-septate	95·6
	1-septate	3·0
	2-septate	1·2
	3-septate	0·2
No. 4	0-septate	89·8
	1-septate	6·0
	2-septate	2·0
	3-septate	2·2

Subcultures of these isolations, when grown on the standard synthetic medium, all developed the characteristic 'indigo blue' ring after ten days, and all showed a predominance of microconidia. These observations on first subcultures of fresh isolations are in entire agreement with those made on other wild strains of *F. oxysporum* as already described. They further confirm the value of the standard synthetic medium (Gogoi, 1949) in the diagnosis of this oil palm strain. A further point of interest was that all four isolations showed sectoring of a rather inconspicuous kind in the first subcultures on the standard synthetic medium. This raises the interesting

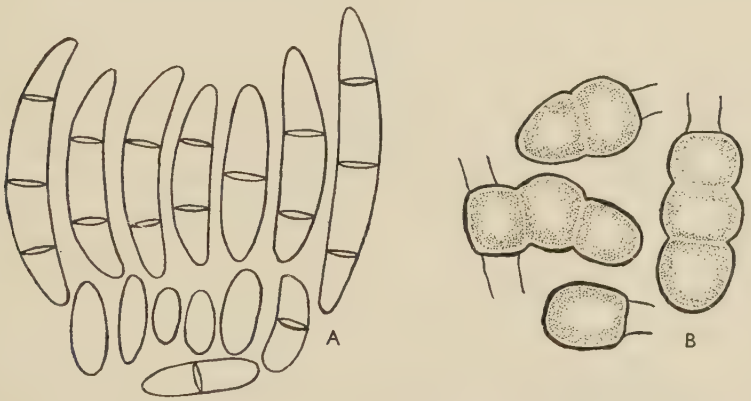
possibility that the oil palm wilt organism may mutate in the necrosed vessels of the host.

Comparison of strains from banana and palm

The two isolations of *F. oxysporum cubense* from the banana wilt disease showed characters closely comparable to those of *F. oxysporum* from the oil palm. On the standard medium, the aerial mycelium was luxuriant and the 'indigo blue' pigmentation was always in the form of a ring. Microconidia and chlamydospores were abundant, macroconidia were not observed after two weeks' growth. Reciprocal inoculation experiments on palm and banana are desirable to ascertain the range of pathogenicity of strains isolated from the two hosts.

Other species of Fusarium *associated with necrosed vascular strands of the oil palm*

Of the thirty-five isolations of *Fusarium* considered here, four were found to be *F. solani*, Text-fig. 2 (isolated from adult palms showing wilting) and one was a strain of *F. dimerum*; this was isolated from an 18-month-old seedling palm showing typical vascular necrosis.



Text-fig. 2. × 1000. A, a group of conidia of *F. solani*; B, a group of chlamydospores of *F. solani*.

The presence of other species of *Fusarium* in necrotic vascular strands of the oil palm is a matter of some interest, i.e. *F. oxysporum* is not the only associated species. Further mycological investigations and tests of the pathogenicity of these species are desirable.

IV. Discussion

The vascular wilt disease of the oil palm, caused by *F. oxysporum*, is a disease recently recorded for the first time. This disease is now recognized to be one of the major problems in the oil palm plantations of West and Central Africa. From the record of the isolations which came into my hands from different parts of Africa during the years 1947 and 1948, it is clear that the

disease is widely spread and is likely to be the subject of investigations by pathologists working in different regions. It was therefore considered desirable to have some standard account of these strains with which new isolations could be compared. An attempt has therefore been made to give an account of the organism as grown on simplified media. The synthetic medium selected has, in fact, proved of greater value in distinguishing the wild and the mutant types than natural media such as a potato decoction agar.

The observations described above, show that the twenty-eight isolations of *F. oxysporum* from the oil palm form a close assemblage of strains. Broadly speaking, freshly isolated strains of *F. oxysporum* from necrosed vascular strands are characterized by a great preponderance of microconidia over macroconidia, the latter being typically 3-septate and very slightly curved, and by the formation of an 'indigo blue' pigment in the standard medium. However, not all of the strains isolated were identical. This raises the question of the rate and extent of mutation under natural conditions and of the pathogenicity of the different strains. Some of these questions must of necessity be the subject of work in palm-growing regions.

The several isolations showed slight differences in respect of the intensity of pigmentation in the standard medium, in the presence or absence of sclerotia and chlamydospores, and in the degree of septation and size of conidia. But in a survey of the twenty-eight isolations it was not found possible to subdivide them into rigid groups. Thus far the number of strains isolated from diseased oil palms is admittedly small, but, the data so far obtained show a considerable difference to those relating to banana wilt disease. Thus Brandes (1919) and Reinking (1927) observed the presence of different strains in their isolations of *F. oxysporum cubense*. Hansford (1926), however, working on a larger scale isolated 300 strains of the section *Elegans*. Of these, 100 were obtained from plant suffering from vascular wilt disease and 200 strains from various sources, soil, plant debris, etc. Both the pathogenic and saprophytic strains showed a wide range of variation in their morphological and cultural characters. These strains were therefore subdivided into five groups according to the size, shape and septation of conidia, sporulation in pionnotes and sporodochia, etc. Further work along similar lines by Ward (1930) indicated that at least four physiologically different strains of *Fusarium* were pathogenic to banana. Wardlaw (1931) made a detailed study of six cultures selected from thirty-six isolations of *F. oxysporum cubense*. When grown on various synthetic media the strains showed important differences in the several cultural characters. From these several observations, the indications are that various strains showing morphological and cultural differences are pathogenic to the banana. Whether a comparable result will be found when the investigations of palm wilt is put on a broader footing remains to be seen. So far, the strains isolated are, as noted above, closely comparable.

The two isolations of *F. oxysporum cubense* from banana wilt disease in the Cameroons showed characters comparable to those of the palm strains when grown on the standard synthetic medium. In view of this similarity, it will be of both academic and practical interest to ascertain the range of

pathogenicity of strains by reciprocal inoculation experiments on the two hosts.

Reinking and Manns (1933, 1934) and Reinking (1934), following the classification of Waksman (1917), described some tropical American soil *Fusaria* as *soil inhabitants* and others as *soil invaders*. This conception has been extended by Garrett (1938, 1944) as follows: 'The *soil inhabitants* are considered to be primitive or unspecialized parasites with a wide host range; these fungi are distributed throughout the soil, and their parasitism appears to be incidental to their saprophytic existence as members of the general soil microflora. The *soil invaders*, to which class the majority of the root-infecting fungi seem to belong, are more highly specialized parasites; the presence of such fungi in the soil is generally closely associated with that of their host plants. In the continued absence of a host plant, such fungi die out in the soil, owing to their inability to compete with the soil saprophytes for an existence on non-living organic matter. This close association between the *soil invaders* and their host plants thus seems to be enforced by the competition of the general soil microflora.' Since the banana in Central America is an introduced crop, it was not unreasonable that *F. oxysporum cubense* should be regarded as a *soil invader*. But in the case of the oil palm, which is indigenous in West and Central Africa, it might well be that *F. oxysporum* is a *soil inhabitant* which has acquired a particular pathogenicity to the oil palm. In this connexion, as a matter of scientific interest, the value of inoculation experiments with different isolations on different hosts may again be stressed.

While the majority of the strains isolated from necrosed vascular strands of the oil palm proved to be *F. oxysporum*, some isolations of other species were also obtained, viz. *F. solani* and *F. dimerum*. As these are common tropical soil fungi, it would be a matter of interest to know if they also are true parasites, or whether they are merely secondary and saprophytic. *F. dimerum* was, in fact, isolated from necrosed vascular strands in an eighteen-month-old seedling.

In addition to the range of variation encountered in the collection of isolations, evidence of mutation has been obtained in the course of these investigations. In some instances, small variations in which the variants differed very slightly from the parent form in morphological and cultural features were obtained. But, in one instance, as the results of progressive subculturing of a wild strain (strain W), a mutant was obtained which differed notably from the parent in its morphological and physiological characters. This mutant tended to replace the parent form during routine culture transfers. The parent form, however, was recovered without difficulty from the master culture. As a fact of experience, it has been found useful to use young cultures (eight to ten days old) for subcultures, to limit the occurrence of mutants.

Although there were instances of the occurrence of variants or mutants in synthetic culture media, the wild strain maintained its original characters for a considerable period of time (six months) when subcultured from soil to soil. This is a matter of some practical importance, and is in keeping with the known fact that pathogenic strains of *Fusarium* may persist in the soil over long periods.

V. Summary

1. An account is given of strains of *Fusarium oxysporum* isolated from the necrosed vascular strands of the oil palm (*Elaeis guineensis*) from West and Central Africa and from banana wilt disease in the Cameroons.

2. The cultural characters of twenty-eight isolations from the oil palm on a standard synthetic medium are discussed. The isolations were found to form a close assemblage of strains.

3. The growth of a wild strain (strain W) and its mutant form and a few other strains of *F. oxysporum* from the oil palm and strains from the banana was compared in the synthetic and natural potato-dextrose agar. The standard synthetic medium was found more useful than the natural medium in distinguishing the mutant form from the parent.

4. Evidence of mutation in the wild strains is given. The mutant forms may show small variations or may differ from the parent to a great extent morphologically and biochemically, these characters being retained. Cultural tests by which a mutant form may be recognized and compared are also indicated.

5. The development of the pigment was found to be affected by the carbon:nitrogen ratio in the medium. The same pigment may be either blue or red according to the alkalinity or acidity in the medium. Acid reaction is not always necessary for pigment formation. The pigment is diffused in the medium.

6. It has been shown that the wild strain maintains its original characters for a considerable period of time in soil culture.

7. The isolations of *F. oxysporum cubense* from the banana wilt disease showed characters closely comparable to those of *F. oxysporum* from the oil palm when grown in the standard synthetic medium and in steamed rice grains.

8. The fact that other species of *Fusarium*, viz. *F. solani* and *F. dimerum*, have also been isolated from necrosed vascular strands shows that *F. oxysporum* may not be the only pathogenic species associated with palm wilt disease.

The writer wishes to convey her thanks to Prof. C. W. Wardlaw for suggesting this work and for his helpful criticism and continued guidance during its progress; and to Mr E. Ashby and Mr P. Dawes for the preparation of photographs.

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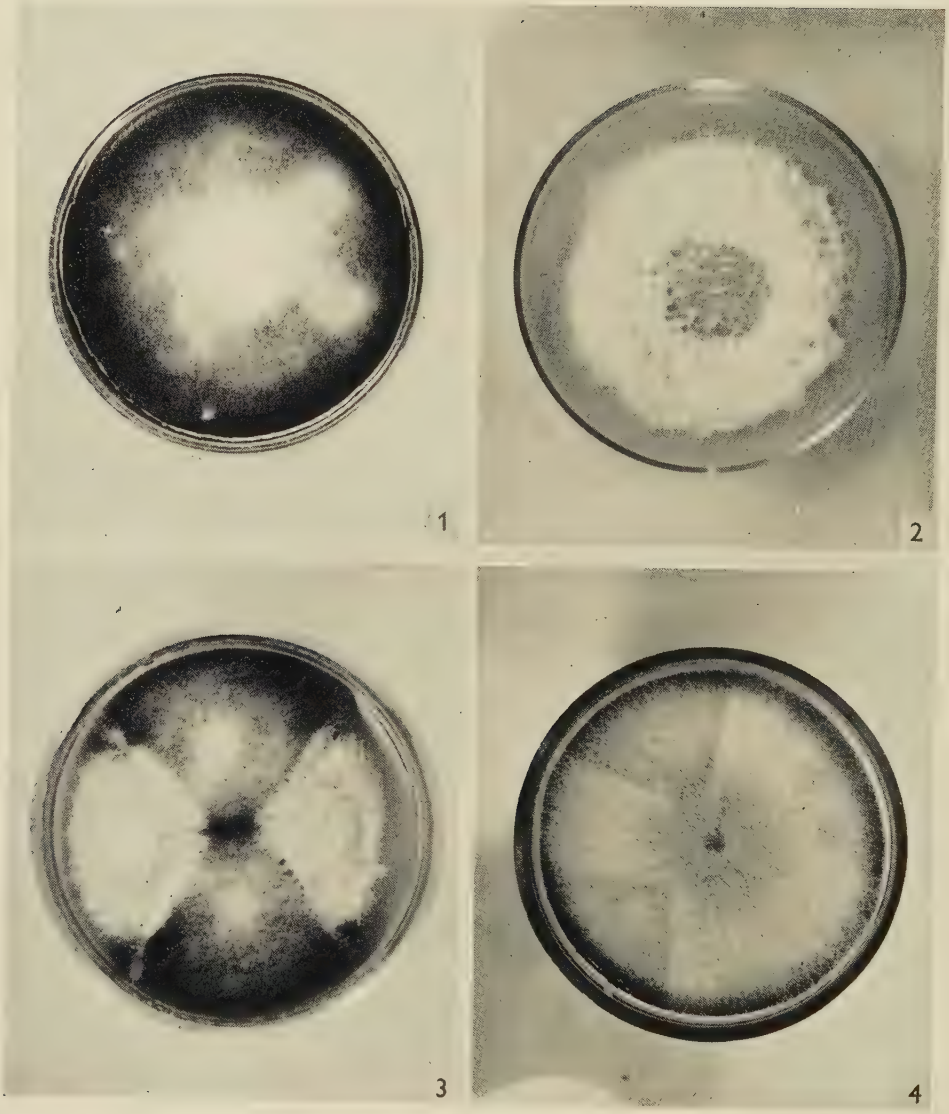
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EXPLANATION OF PLATE 12

Fig. 1. Culture of the wild strain in standard synthetic medium showing development of the pigment.
Fig. 2. Culture of a mutant form in standard synthetic medium showing no pigment development.
Fig. 3. Cultures of the wild strain and a mutant form (grown on the same plate) in standard synthetic medium showing development and no development of the pigment.
Fig. 4. Culture of the wild strain showing sectorings.

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SOME NUTRITIONAL CONDITIONS AFFECTING SPORE PRODUCTION BY *TRICHODERMA VIRIDE* PERS. EX FRIES

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It had been noticed, in the course of experiments on the production of the antibiotics viridin and gliotoxin by *Trichoderma viride*, that the composition of the medium had a profound effect on the production of spores (conidia). The amount of sporulation appeared to be to a considerable degree unrelated to the amount of vegetative mycelium formed. Since production of large inocula for mass-cultures was of some importance, a number of experiments were set up to follow the relation between nutrition and conidial production more closely. These experiments have served to demonstrate the existence of an interesting and complex series of interrelationships between the various nutritional factors studied; the relevant data are presented and discussed below.

Materials and methods

Five strains of *Trichoderma viride* were used. Four of these were of the diffusely-growing, non-tufted type; these included two gliotoxin-producing strains, 12 and 211 (Brian and Hemming, 1945) and two viridin-producing strains, 10 and 213 (Brian, Curtis, Hemming and McGowan, 1946). In addition, a typical tufted form was included: this was strain 41, received from the National Collection of Type Cultures as *T. koningi* Oudem. (N.C.T.C. 3676). Bisby (1939) regards *T. koningi* Oudem. as a synonym of *T. viride* Pers. ex Fries.

The basic media used in these experiments were Weindling and Czapek-Dox. Minor elements were added to each of these media in the form of a concentrate to give the following final concentrations: iron, 0·2 μg./ml.; manganese, 0·02 μg./ml.; zinc, 0·2 μg./ml.; copper, 0·03 μg./ml.; molybdenum, 0·02 μg./ml. These concentrations were based on those recommended by Steinberg (1935) for *Aspergillus niger*. The detailed compositions of media and of the minor element concentrate have been previously described (Brian, Curtis and Hemming, 1946). The pH of the media was adjusted, where required, with hydrochloric acid or potassium hydroxide. Chemicals of analytical grade were used throughout.

The experimental cultures were set up in triplicate on 30 ml. lots of medium in 100 ml. Pyrex flasks, each flask being inoculated with approximately 0·5 million spores suspended in 1 ml. distilled water and incubated at 25° C. in the dark.

Periodical visual assessments of the degree of sporulation were made, together with observations of the type of growth. The following types of growth were encountered:

'*Aerial*' (A): strong surface felts with abundant aerial mycelium; this type of growth was essential for abundant sporulation.

'*Slimy*' (S): waterlogged felts, with the mycelium mainly submerged; this was particularly characteristic of some strains of *T. viride* and was associated with absence of sporulation.

'*Edge*' (E): occasionally some aerial mycelium was formed on the glass where 'slimy' felts touched the edge of the culture flask, sporulation usually then taking place.

After seven to twelve days' growth (differing somewhat in different experiments) the contents of each culture flask were decanted into a large flask containing 270 ml. water and shaken vigorously. The spore density in each of three samples from each resulting suspension was determined by means of a Thoma haemacytometer. Data are presented as the mean number of spores counted in eighty small haemacytometer squares for each treatment. If this figure is multiplied by fifteen millions the mean number of spores per flask is obtained. The data presented in the tables that follow have been much condensed, the visual observations on sporulation being entirely omitted. It was found in general that those media on which sporulation was first visible were those supporting the finally greater populations of spores and that visual estimates of spore numbers agreed with the haemacytometer counts in most cases.

Experimental results

(*a*) *Effect of initial* pH

In this experiment the glucose (2·5%) and salts were autoclaved separately, mixed just before use and steamed lightly for a short time to avoid breakdown of the glucose in solutions of high pH. The salt solutions were adjusted to pH values of 3·0, 5·0, 8·0 and 9·0. As will be seen from Table 1 the pH changed considerably during sterilization. Spore counts were made after eight days' growth.

There is a clear response to initial pH, varying between the strains and between the two media. There is a general tendency for sporulation to be most vigorous in media of intermediate or high pH. The lowest pH was unsuitable for all strains, sporulation being negligible and growth of the slimy type. The highest pH tested (7·0) was optimal for strains 10 and 41.

(*b*) *Effect of glucose concentration*

Weindling and Czapek-Dox media, both at pH 5·0, were made up with glucose concentrations ranging from 0·1 to 10·0%. Spore counts were made after seven days' growth (Table 2). Strain 41 did not sporulate under the conditions of this experiment, growth being of the slimy type throughout. Strains 10, 12 and 211 showed optima, on both basic media, at intermediate dextrose concentrations. With strain 213 sporulation was optimal with the higher sugar concentration.

(c) Relative proportions of magnesium sulphate, potassium phosphate and a nitrogen source (ammonium tartrate or sodium nitrate)

These three constituents were chosen for variation as being physiologically the most important major mineral constituents of the medium. Media were made up with three different levels of the three constituents in all possible combinations. The following key indicates the compositions of the media listed in Tables 3 and 4:

Weindling	Czapek-Dox
A_1 = 0·4 g./l. ammonium tartrate	A_1 = 0·4 g./l. $NaNO_3$
A_2 = 2·0 g./l. ammonium tartrate	A_2 = 2·0 g./l. $NaNO_3$
A_3 = 10·0 g./l. ammonium tartrate	A_3 = 10·0 g./l. $NaNO_3$
B_1 = 0·4 g./l. KH_2PO_4	B_1 = 0·2 g./l. KH_2PO_4
B_2 = 2·0 g./l. KH_2PO_4	B_2 = 1·0 g./l. KH_2PO_4
B_3 = 10·0 g./l. KH_2PO_4	B_3 = 5·0 g./l. KH_2PO_4
C_1 = 0·2 g./l. $MgSO_4.7H_2O$	C_1 = 0·1 g./l. $MgSO_4.7H_2O$
C_2 = 1·0 g./l. $MgSO_4.7H_2O$	C_2 = 0·5 g./l. $MgSO_4.7H_2O$
C_3 = 5·0 g./l. $MgSO_4.7H_2O$	C_3 = 2·5 g./l. $MgSO_4.7H_2O$

The media were adjusted approximately to pH 5·0; all contained 2·5 % glucose. The results of variations on the Weindling formula are shown in Table 3 (nine days' growth), variations on the Czapek-Dox formula in Table 4 (ten days' growth).

The level of nitrogen had the most striking effect. Strains 10, 12 and 41 only sporulated appreciably on the media with the lowest level of nitrogen: the effect was less well marked with strains 211 and 213. It is of interest to note that vigour of growth did not run parallel with sporulation in these experiments; the low-nitrogen media produced very thin felts, increasing in thickness as the nitrogen level was raised. The phosphate level had little effect. With strains 211 and 213 the magnesium sulphate level was of some importance. On the Czapek-Dox medium, at the highest nitrogen levels, high magnesium sulphate levels are best; at the middle (normal) nitrogen level, with strain 213 in particular, high $MgSO_4$ is preferable. On the Weindling medium this only shows up clearly with strain 211.

(d) Effect of addition of calcium chloride or lithium chloride to media

Calcium chloride and lithium chloride were added to the standard Weindling and Czapek-Dox media in concentrations between 0·001 and 1·0 %. The media were adjusted to pH 5; all contained 2·5 % glucose. Results (seven days' growth) are shown in Table 5.

On the Weindling medium calcium chloride and lithium chloride were highly stimulatory (in suitable concentrations) with strains 211 and 213. On Czapek-Dox calcium chloride was stimulatory with strains 10, 211, 41 and 12. Lithium chloride was stimulatory only with strains 211 and 213. The highest concentration of lithium chloride (1·0 %) was definitely toxic, reducing the vigour of growth noticeably. The optimum concentration of calcium chloride was in the range 0·01–0·1 %.

Table 1. *Effect of initial* pH *of medium*

Medium	Spore count Strain 10	12	41	211	213	Type of growth Strain 10	12	41	211	213
Weindling										
pH 2·1	0	0	0	2	0	S	S	S	S	S
4·5	0	0	0	8	24	S	S	S	A	A
6·2	12	24	0	56	76	A	A	S	A	A
6·8	34	34	17	73	70	A	A	E	A	A
7·0	67	19	18	29	44	A	A	E	A	A
Czapek-Dox										
pH 1·7	0	2	0	0	2	S	S	S	S	S
4·0	5	13	0	49	20	E	S	S	A	A
6·1	0	21	0	50	23	S	A	E	A	A
6·8	20	0	1	32	26	A	S	E	A	A
7·0	22	0	2	48	3	A	S	E	A	S

Table 2. *Effect of glucose concentration*

Medium	Glucose (%)	Spore count Strain 10	12	41	211	213	Type of growth Strain 10	12	41	211	213
Weindling	0·1	8	3	0	41	39	A	S	S	A	A
	0·5	13	34	0	147	70	A	A	S	A	A
	1·0	12	7	0	137	74	A	A	S	A	A
	2·5	1	0	0	181	75	S	S	S	A	A
	10·0	0	0	0	70	99	S	S	S	A	A
Czapek-Dox	0·1	1	2	0	62	10	E	A	S	A	A
	0·5	14	1	0	149	11	A	S	S	A	A
	1·0	1	0	0	293	19	S	S	S	A	A
	2·5	0	0	0	238	23	S	S	S	A	A
	10·0	0	1	0	130	94	S	S	S	A	A

Table 3. *Effect of varying levels of ammonium tartrate (A), potassium dihydrogen phosphate (B) and magnesium sulphate (C) in Weindling medium*

Composition	Spore count Strain 10	12	41	211	213	Type of growth Strain 10	12	41	211	213
$A_1B_1C_1$	4	3	49	36	70	A	A	E	A	A
$A_1B_1C_2$	4	13	52	102	86	A	A	E	A	A
$A_1B_1C_3$	15	3	3	49	83	A	A	E	A	A
$A_1B_2C_1$	8	5	51	26	79	A	A	E	A	A
$A_1B_2C_2$	16	5	10	30	96	A	A	E	A	A
$A_1B_2C_3$	15	3	113	111	59	A	A	E	A	A
$A_1B_3C_1$	16	10	93	63	121	A	A	E	A	A
$A_1B_3C_2$	8	2	46	57	133	A	A	E	A	A
$A_1B_3C_3$	0	2	126	50	171	A	A	E	A	A
$A_2B_1C_1$	0	0	0	10	25	S	S	S	A	A
$A_2B_1C_2$	0	0	0	94	35	S	S	S	A	A
$A_2B_1C_3$	0	0	0	52	25	S	S	S	A	A
$A_2B_2C_1$	0	0	0	11	35	S	S	S	A	A
$A_2B_2C_2$	0	0	0	88	44	S	S	S	A	A
$A_2B_2C_3$	0	0	0	62	56	S	S	S	A	A
$A_2B_3C_1$	0	0	0	31	59	S	S	S	A	A
$A_2B_3C_2$	0	0	0	42	111	S	S	S	A	A
$A_2B_3C_3$	0	0	0	23	81	S	S	S	A	A
$A_3B_1C_1$	1	2	5	9	27	S	A	E	A	A
$A_3B_1C_2$	1	1	0	25	15	S	A	S	A	A
$A_3B_1C_3$	5	6	0	50	26	S	A	S	A	A
$A_3B_2C_1$	0	2	0	8	46	S	A	S	A	A
$A_3B_2C_2$	2	3	0	19	32	S	A	S	A	A
$A_3B_2C_3$	1	2	0	39	38	S	A	S	A	A
$A_3B_3C_1$	0	0	0	13	76	S	A	S	A	A
$A_3B_3C_2$	1	1	0	18	42	S	A	S	A	A
$A_3B_3C_3$	1	7	0	35	44	S	A	S	A	A

Table 4. *Effect of varying levels of sodium nitrate (A), potassium dihydrogen phosphate (B) and magnesium sulphate (C) in Czapek-Dox medium*

Composition	Spore count, Strain 10	12	41	211	213	Type of growth, Strain 10	12	41	211	213
$A_1B_1C_1$	43	121	0	81	75	A	A	S	A	A
$A_1B_1C_2$	23	40	0	106	146	A	A	S	A	A
$A_1B_1C_3$	18	50	0	25	49	A	A	S	A	A
$A_1B_2C_1$	38	9	0	56	95	A	A	S	A	A
$A_1B_2C_2$	32	13	0	45	84	A	A	S	A	A
$A_1B_2C_3$	18	31	0	61	61	A	A	S	A	A
$A_1B_3C_1$	16	6	0	54	22	A	A	S	A	A
$A_1B_3C_2$	19	14	0	22	69	A	A	S	A	A
$A_1B_3C_3$	26	22	0	47	45	A	A	S	A	A
$A_2B_1C_1$	2	2	0	53	12	S	S	S	A	A
$A_2B_1C_2$	1	0	0	132	117	S	S	S	A	A
$A_2B_1C_3$	2	1	0	23	57	S	S	S	A	A
$A_2B_2C_1$	6	6	0	61	3	S	S	S	A	A
$A_2B_2C_2$	4	5	0	162	150	S	S	S	A	A
$A_2B_2C_3$	2	3	0	60	259	S	S	S	A	A
$A_2B_3C_1$	3	8	0	21	4	S	S	S	A	A
$A_2B_3C_2$	4	4	0	23	256	S	S	S	A	A
$A_2B_3C_3$	1	1	0	61	183	S	S	S	A	A
$A_3B_1C_1$	1	0	0	4	6	S	A	S	A	A
$A_3B_1C_2$	0	0	0	8	5	S	A	S	A	A
$A_3B_1C_3$	1	0	0	32	33	S	A	S	A	A
$A_3B_2C_1$	0	0	0	1	0	S	S	S	A	A
$A_3B_2C_2$	1	0	0	1	10	S	A	S	A	A
$A_3B_2C_3$	0	0	0	11	17	S	A	S	A	A
$A_3B_3C_1$	0	1	0	1	2	S	A	S	A	A
$A_3B_3C_2$	0	0	0	3	4	S	A	S	A	A
$A_3B_3C_3$	1	0	0	20	19	S	A	S	A	A

Table 5. *Effect of calcium chloride and lithium chloride supplements*

Medium	Spore count, Strain 10	12	41	211	213	Type of growth, Strain 10	12	41	211	213
Weindling										
Normal	0	1	0	28	47	S	S	S	A	A
+0·001% $CaCl_2$	2	0	0	202	45	S	S	S	A	A
+0·01% $CaCl_2$	3	0	0	170	164	S	S	S	A	A
+0·1% $CaCl_2$	4	8	0	226	274	S	A	S	A	A
+1·0% $CaCl_2$	0	1	0	159	34	S	S	S	A	A
+0·001% $LiCl_2$	0	2	0	34	32	S	S	S	A	A
+0·01% $LiCl_2$	1	1	0	131	102	S	S	S	A	A
+0·1% $LiCl_2$	3	1	0	26	6	S	S	S	A	A
+1·0% $LiCl_2$	0	0	0	0	2	S	S	S	A	A
Czapek-Dox										
Normal	5	0	0	84	6	S	S	S	A	A
+0·001% $CaCl_2$	3	0	0	189	0	S	S	S	A	A
+0·01% $CaCl_2$	16	0	0	227	1	E	S	S	A	A
+0·1% $CaCl_2$	17	0	6	99	5	E	S	E	A	A
+1·0% $CaCl_2$	12	15	0	89	2	E	A	S	A	A
+0·001% $LiCl_2$	4	1	0	169	3	S	S	S	A	A
+0·01% $LiCl_2$	1	1	1	131	7	S	S	S	A	A
+0·1% $LiCl_2$	0	0	0	108	2	S	S	S	A	A
+1·0% $LiCl_2$	0	0	0	1	0	S	S	S	A	S

(*e*) *Effect of trace elements*

Weindling and Czapek-Dox media were made up to the formulae given, *without* addition of minor elements. To remove trace elements present as impurities, to each litre of medium 15 g. of calcium carbonate were added and the mixture was autoclaved for 20 min. (Steinberg, 1935). This was then filtered while hot through ashless paper and distributed among the culture flasks. The trace elements iron, manganese, zinc and copper (as sulphates) and molybdenum (as potassium molybdate) were added in the standard concentrations mentioned previously, singly and in all combinations. All glassware used was treated with chromic acid cleaning mixture and thoroughly washed in glass-distilled water; all solutions were made up with glass-distilled water. The resulting media had a pH of 6·4; no adjustment was made. Results with Weindling medium are given in Table 6 (nine days' growth), with Czapek-Dox (twelve days' growth) in Table 7.

Growth was negligible in the media with no minor elements added. In general, growth appeared to be best in the two and three element combinations containing iron but not zinc, in the four element combinations containing iron and in the five element combination. The amount of sporulation did not follow the vigour of growth at all closely.

Table 6. *Effect of minor elements in Weindling medium*

Minor elements	Spore count, Strain					Type of growth, Strain				
	10	12	41	211	213	10	12	41	211	213
—	1	0	0	3	51	S	S	S	A	A
Fe	10	1	0	124	152	A	S	S	A	A
Cu	2	0	0	41	41	S	S	S	A	A
Zn	2	0	0	16	44	S	S	S	A	A
Mn	2	1	0	67	75	S	S	S	A	A
Mo	8	0	0	28	56	S	S	S	A	A
Fe Mn	49	1	0	147	201	A	S	S	A	A
Fe Cu	19	1	0	157	166	A	S	S	A	A
Fe Zn	32	1	0	161	171	A	S	S	A	A
Fe Mo	77	0	0	101	100	A	S	S	A	A
Mn Cu	48	0	0	60	24	A	S	S	A	A
Mn Zn	2	0	0	63	66	S	S	S	A	A
Mn Mo	1	0	0	42	40	A	S	S	A	A
Cu Zn	1	0	0	17	41	S	S	S	A	A
Cu Mo	2	0	0	21	19	S	S	S	A	A
Zn Mo	1	0	0	27	34	S	S	S	A	A
Fe Mn Zn	34	1	0	110	246	A	S	S	A	A
Fe Mn Cu	44	1	0	166	267	A	S	S	A	A
Fe Mn Mo	41	2	0	158	201	A	S	S	A	A
Fe Zn Cu	6	0	0	98	111	A	S	S	A	A
Fe Zn Mo	8	0	0	89	46	A	S	S	A	A
Fe Cu Mo	16	0	0	86	37	A	S	S	A	A
Mn Zn Cu	1	0	0	44	30	S	S	S	A	A
Mn Zn Mo	1	0	0	28	80	S	S	S	A	A
Zn Cu Mo	18	0	0	31	16	A	S	S	A	A
Cu Mo Mn	2	1	0	57	31	S	S	S	A	A
Fe Mn Zn Cu	34	2	0	128	149	A	S	S	A	A
Fe Mn Zn Mo	48	2	0	168	141	A	S	S	A	A
Fe Zn Cu Mo	14	2	0	104	65	A	S	S	A	A
Mn Zn Cu Mo	3	0	0	74	63	S	S	S	A	A
Fe Mn Cu Mo	28	2	0	74	237	A	S	S	A	A
Fe Mn Zn Cu Mo	47	3	0	126	284	A	S	S	A	A

Table 7. *Effect of minor elements in Czapek-Dox medium*

Minor elements	Spore count Strain 10	12	41	211	213	Type of growth Strain 10	12	41	211	213
—	4	1	2	1	25	A	S	A	A	A
Fe	140	3	37	163	73	A	A	A	A	A
Cu	84	2	5	14	55	A	S	A	A	A
Zn	5	1	5	28	32	A	S	A	A	A
Mn	6	6	3	8	29	A	A	A	A	A
Mo	5	3	5	9	22	A	A	A	A	A
Fe Mn	179	2	72	92	87	A	A	A	A	A
Fe Cu	154	1	51	106	12	A	S	A	A	A
Fe Zn	13	2	11	222	39	S	S	A	A	A
Fe Mo	148	3	152	171	44	A	S	A	A	A
Mn Cu	7	2	3	16	76	A	S	A	A	A
Mn Zn	3	0	10	13	60	A	S	A	A	A
Mn Mo	9	2	9	16	13	A	S	A	A	A
Cu Zn	9	1	6	121	60	S	S	A	A	A
Cu Mo	23	0	2	7	31	A	A	A	A	A
Zn Mo	5	2	2	20	53	A	S	A	A	A
Fe Mn Zn	22	1	9	187	85	E	S	A	A	A
Fe Mn Cu	169	7	24	86	104	A	A	A	A	A
Fe Mn Mo	14	13	150	346	134	A	E	A	A	A
Fe Zn Cu	1	2	26	155	137	S	S	A	A	A
Fe Zn Mo	8	4	17	65	77	S	S	A	A	A
Fe Cu Mo	11	6	15	53	34	E	A	A	A	A
Mn Zn Cu	3	2	3	33	48	A	S	A	A	A
Mn Zn Mo	6	3	2	13	34	A	A	A	A	A
Zn Cu Mo	7	0	4	12	60	A	S	A	A	A
Cu Mo Mn	13	2	10	21	28	A	A	A	A	A
Fe Mn Zn Cu	4	2	11	202	90	S	S	A	A	A
Fe Mn Zn Mo	27	2	10	84	99	E	S	A	A	A
Fe Zn Cu Mo	8	1	3	74	76	E	A	A	A	A
Mn Zn Cu Mo	5	6	1	6	43	A	A	A	A	A
Fe Mn Cu Mo	92	2	73	170	141	A	A	A	A	A
Fe Mn Zn Cu Mo	15	2	34	89	50	A	S	A	A	A

The following points should be noted:

(i) The effect of iron is the most marked, no combination in which iron is omitted being optimal for sporulation. Several strains gave excellent sporulation when iron alone was added.

(ii) There was no clear evidence that any minor element other than iron was essential, though with several strains, notably with strain 213, sporulation was improved by combination of various minor elements with iron. The normal green colour of the spores was not developed in the absence of copper; spores produced on copper-deficient media were yellow. The absence of any clear-cut response to other minor elements may well have been due to inefficient removal from the basal medium, though in the hands of Steinberg (1935) the technique was quite satisfactory.

(iii) There is evidence that in the case of strains 10 and 41 addition of zinc to Czapek-Dox medium reduced sporulation in combinations with other minor elements. The effect was not seen on Weindling medium.

(iv) There is a considerable variation in response between strains. The addition of minor elements had little effect on strain 12, under the conditions of the experiment, on either medium, and had no effect on strain 41 on

Weindling medium. The other strains showed clearer responses. Strain 211 produced optimal sporulation, on both media, with a single addition of iron only. Strain 213 showed optimal responses only with combination of at least three minor elements, on both media. Strain 10 gave optimal sporulation with a single iron addition on Czapek-Dox but needed more elaborate combinations on Weindling.

(v) The effects of the various minor elements are interrelated, so that several combinations may give optimal sporulation. The minor element demands vary with different basal media. It is thus not possible to define the minor element requirements of *T. viride* quantitatively without reference to strain, basal medium and the nature of the minor elements present.

(*f*) *The relation between sporulation and mycelial growth*

Several cases have been mentioned where sporulation appeared to be unrelated to vigour of growth. Quantitative data have been obtained in two experiments. In these, in addition to spore counts, mean dry weights of mycelia from six comparable flask cultures were also determined. Table 8 shows the results of an experiment where the concentration of glucose was varied in Weindling and Czapek-Dox media (pH 5·0). The spore counts (ten days' growth) were comparable in general drift with those previously recorded (Table 2), strain 41 scarcely sporulating on any media, intermediate glucose concentrations favouring strain 211 and high glucose concentrations favouring strain 213. It will be seen that the dry weight of mycelium increased with increasing glucose concentration and that there was a general tendency for the spore/mycelium ratio to fall as the sugar concentration was increased.

Table 9 gives the results of an experiment where the concentration of nitrogen source in Weindling and Czapek-Dox media (pH 5·0, 2·5 % glucose) was varied. Again the spore counts (twelve days' growth) generally confirmed previous results (Tables 3 and 4); with some exceptions low or intermediate nitrogen concentrations favoured sporulation. Mycelial dry weight usually increased with increasing nitrogen supply and the spore/mycelium ratio in every case fell with increasing nitrogen.

Discussion

Three main conclusions may now be drawn:

(1) It is not possible to lay down exact optimal conditions for sporulation of the species *T. viride*; the extent of strain variation is so considerable that one can only define optima for the strain or strains under investigation. This is probably true of other fungus species.

(2) The number of nutritional factors affecting sporulation of *T. viride* are very great. These factors must be conceived as acting in a complex web of interactions and the optimum level of any one factor cannot be defined except in relation to the levels of other factors. No attempt has been made, in the present investigation, to analyse at all closely these interactions, but the underlying complexity has been demonstrated.

(3) Conditions favouring vigorous mycelial growth may either lead to increased or decreased sporulation when the latter is defined in terms of

Table 8. *Effect of glucose concentration on spore/mycelium ratio*

Glucose (%)	Strain 41			Strain 211			Strain 213		
	S	M	S/M	S	M	S/M	S	M	S/M
Weindling									
0·1	1	6	0·17	11	8	1·38	8	10	0·80
1·0	1	30	0·03	100	65	1·54	21	75	0·28
10·0	0	240	0	52	287	0·18	56	307	0·18
Czapek-Dox									
0·1	0	24	0	30	8	3·75	10	10	1·00
1·0	0	73	0	154	105	1·47	14	113	0·12
10·0	0	340	0	85	364	0·23	68	526	0·13

Note: S= spore count; M=dry weight of mycelium (mg./flask); S/M=spore/mycelium ratio.

Table 9. *Effect of nitrogen source concentration on spore/mycelium ratio*

Medium	Strain 41			Strain 211			Strain 213		
	S	M	S/M	S	M	S/M	S	M	S/M
Weindling									
$A_1B_2C_2$ *	41	47	0·87	65	55	1·18	71	72	0·99
$A_2B_2C_2$	0	105	0	75	108	0·69	64	167	0·38
$A_3B_2C_2$	0	160	0	43	139	0·31	39	148	0·26
Czapek-Dox									
$A_1B_2C_2$	0	73	0	69	107	0·65	105	138	0·76
$A_2B_2C_2$	0	140	0	154	181	0·85	4	201	0·02
$A_3B_2C_2$	0	140	0	3	243	0·01	1	309	0

* Significance of code as in Table 3.

Note. S=spore count; M=dry weight of mycelium (mg./flask); S/M=spore/mycelium ratio

the number of spores produced in each culture. If sporulation per unit weight of mycelium is considered, it is found that conditions favourable to mycelial growth reduce sporulation. This result is in accordance with the broad conclusion of Klebs (1896, 1900) that conditions favouring vigorous vegetative growth of fungi are unfavourable to production of sexual or asexual spore stages. The mycologist seeking to induce sporulation of a difficult fungus in culture might be advised to use media of low nutritional status. On the other hand, when faced with the practical problem of production of large inocula, where the number of spores in each culture vessel is the criterion rather than the spore/mycelium ratio, it may be necessary to grow the fungus under nutritional conditions optimal for growth.

Summary

1. The effect of various nutrient combinations on production of conidia by five strains of *Trichoderma viride* has been studied, using an objective measure of the amount of sporulation.

2. The strains of *T. viride* differed in their detailed response but the following general tendencies were observed: (*a*) A medium of initial pH 4·0–6·0 was preferable to a higher or lower value. (*b*) Most strains sporulated most vigorously in media in the middle of the range 0·1–10·0 % glucose, though one strain preferred the highest value. (*c*) In a series of media in which magnesium sulphate, nitrogen source (ammonium tartrate or sodium

nitrate) and phosphate were introduced in all possible combinations of three concentration levels, it was found that variation in nitrogen concentration had most significance, a general tendency being observed for sporulation to be most vigorous on media with the lower nitrogen levels; in the media with high nitrogen, a high magnesium sulphate level favoured sporulation; the phosphate level did not appreciably affect sporulation. (*d*) Sporulation of most strains was stimulated by addition of calcium chloride (0·01–0·1 %) but lithium chloride only stimulated sporulation of one strain. (*e*) Iron was found to be essential for optimum growth and sporulation, copper was essential for development of the usual green colour of the spores but other minor elements (zinc, molybdenum and manganese), though improving growth and sporulation on occasion, could not be regarded as essential; zinc decreased sporulation under certain conditions in two strains.

3. Conditions favouring vigorous mycelial growth tended to reduce the number of spores formed per unit weight of mycelium, though the number of spores formed per culture might be increased.

4. There was considerable evidence of interaction between the nutritional factors studied.

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THE DOWNWARD MOVEMENT OF FUNGAL SPORES IN SANDY SOIL

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(With 5 Text-figures)

Discussions of the dilution plate technique of investigating soil fungi often contain references to the possibility of fungal spores being washed through the soil. If spores were easily washed downwards through soil then investigations into the vertical distribution of fungi in soils would be subject to serious errors if the dilution plate technique were used. Errors would be most serious when the upper layers of the soil contained fungi which spored abundantly. So far as I am aware there is no record of experiments to determine whether fungal spores will wash downwards through the soil. Workers who have investigated the possibility of pollen grains being washed downwards through peat reached the conclusion that there is very little movement of pollen grains. The problem of the movement of fungal spores is, however, somewhat different. First, fungal spores are much smaller than pollen grains, and secondly, pollen studies have been mainly concerned with peat or very heavy soils.

During an investigation of the soil fungi of the Breckland, East Anglia, numerous isolations were made from soil samples collected at various depths. From the results obtained it seemed clear that some fungi showed distinct vertical zonation. In an attempt to explain such zonation three fungi with representative distributions were selected for further study. The three fungi were *Penicillium cyclopium*—which occurred abundantly in the upper few centimetres of the soil but was rarely isolated from the lower layers; *Zygorrhynchus vuillemini*—which was only occasionally found in the upper layers but was abundant in the soil below 10 cm.; and *Gliomastix convoluta*—which was isolated from all layers examined. The soil investigated in most detail was formed from stabilized wind-blown sand and was uniform in texture without any distinct layering for at least 1·25 m. If there is a tendency for fungal spores to be washed downwards one would expect them to move most readily in a loose-textured soil of that kind. Experiments were therefore carried out to determine the extent to which the spores of the fungi being examined would wash through the soil.

In order to provide suitable columns of sand for investigation a glass frame was prepared as follows. A back plate of glass 5 by 60 cm. was used, and two side walls 3·5 by 55 cm. cemented to this with canada balsam. The front edges of the side plates were ground to give smooth straight edges. These were coated with vaseline and the front plate 3·5 by 55 cm. placed in position. This was held firmly by short pieces of wood and strong rubber bands as shown in Fig. 1. A plug of sterilized cotton-wool was fitted into the lower end and the column filled with sand. The cotton-wool plug was kept in position by a small bent glass rod. The sand was taken

from the area being examined and, after air-drying, was sifted to remove roots and other organic matter. It was then autoclaved and allowed to dry. No special precautions were taken to keep the column of sand strictly aseptic. After the column had been filled, about 200–300 c.c. of sterile water was allowed to flow through it. This caused the sand to assume a packing comparable with that of the sand in the field after rain. The excess water was allowed to drain off. It is essential that the sand be well packed, otherwise channels develop through which the spores readily wash. A spore suspension of the required fungus was poured carefully on to the top of the sand and then a further amount of sterile water added. Both the spore suspension and the additional water must be poured on slowly to prevent too much disturbance of the upper layers of the sand.

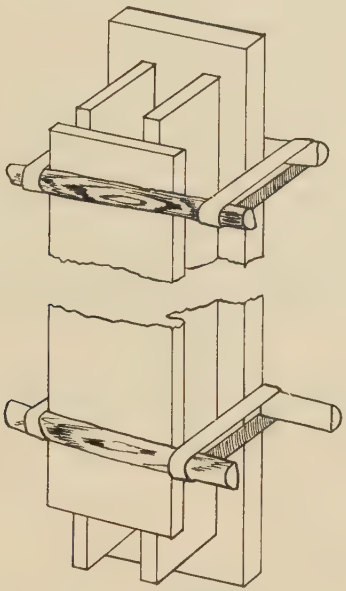

Fig. 1. Arrangement of the plates of glass to form the column. For full description see text.

Preliminary experiments showed that the spores of both *Zygorrhynchus* and *Gliomastix* moved downwards through the sand. Further experiments were therefore carried out using different amounts of sterile water. In the experiment with *Gliomastix* recorded in Fig. 3 the procedure was as follows: the column was set up and about 10 c.c. of a suitable spore suspension prepared; 2 c.c. of this was poured on to the top of the column and followed by 100 c.c. of sterile water. After the excess water had drained off the column was placed horizontally and the front plate carefully removed. The outer part of the sand was discarded and samples taken from the centre of the sand at various depths. These samples were transferred to flasks of sterile water and dilution counts made in the normal way. Immediately after the first samples had been taken the column was refilled and a further 2 c.c. of the original spore suspension used for a second

experiment, in which 200 c.c. of washing water were used. A third experiment was then carried out still using the same spore suspension, but with 600 c.c. of washing water. The sample of sand for the dilution count was weighed wet before being transferred to the flask. In the early experiments the sand was recovered after the dilution-plates had been poured and the dry weight of the sand obtained. The figures for the various samples were then compared on this dry weight basis. It soon became clear however that, provided the column was left long enough for the excess water to drain away, the wet weight figure was sufficiently accurate.

After each experiment the glass of the column was washed and then sponged with 95 % alcohol. No other sterilization was found necessary. The number of contaminating colonies which appeared was remarkably small, and as the counts were being made on specific fungi the contaminants were readily recognized and neglected.

Fig. 2 shows the results obtained with the *Zygorrhynchus*, and in Fig. 3 are the results of experiments with *Gliomastix*. After the preliminary experiments it was apparent that spores of this fungus were likely to be washed right through the column with large quantities of water, consequently 1 c.c. of the water which came from the column towards the end of the experiment was plated out as well. The counts of the spores which had washed through the column and come out in the drainage water are shown at the bottom of Fig. 3. Fig. 4 gives the results of a representative experiment with *Penicillium*. In Fig. 5 are the results from a trial in which a suspension of a mixture of *Gliomastix* and *Penicillium* spores was used.

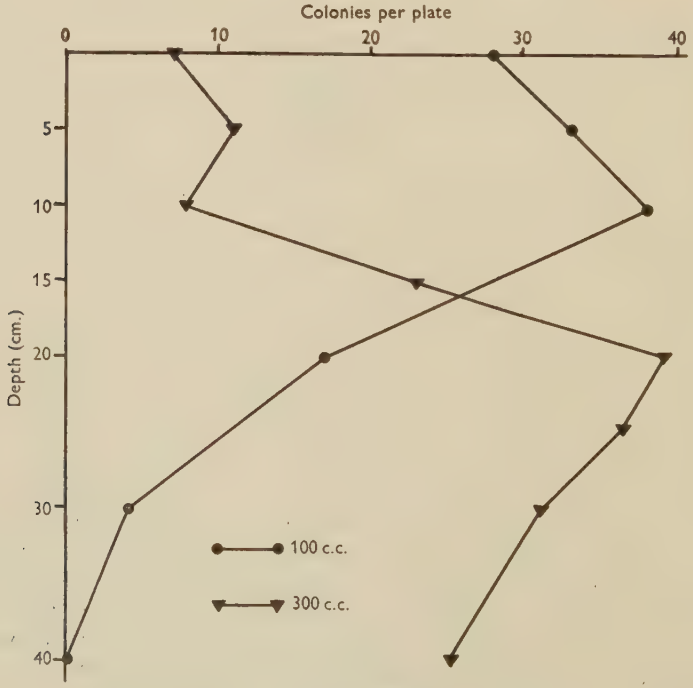

Fig. 2. Results obtained with *Zygorrhynchus* using two different amounts of water.

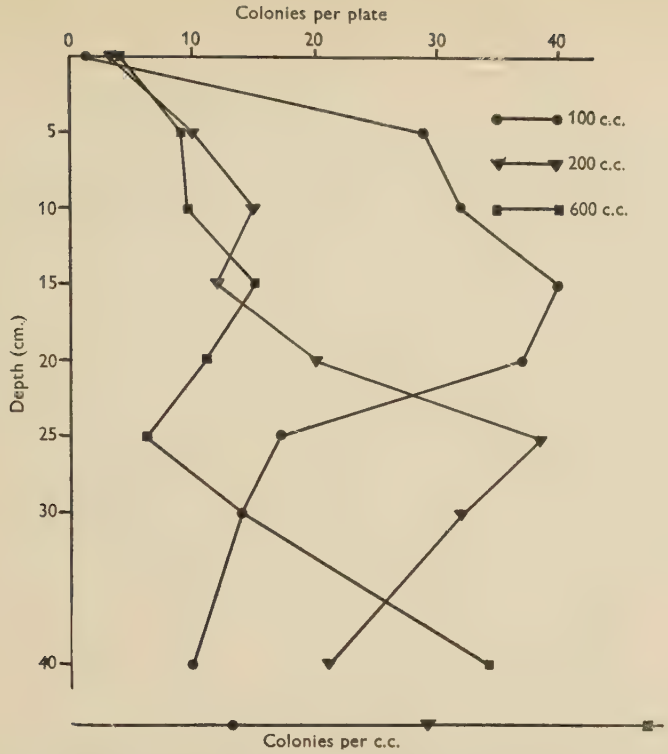

Fig. 3. Results of experiments with *Gliomastix*, using three different amounts of water. The values given at the bottom of the figure represent the extent to which the spores were washed through the column and appeared in the drainage water.

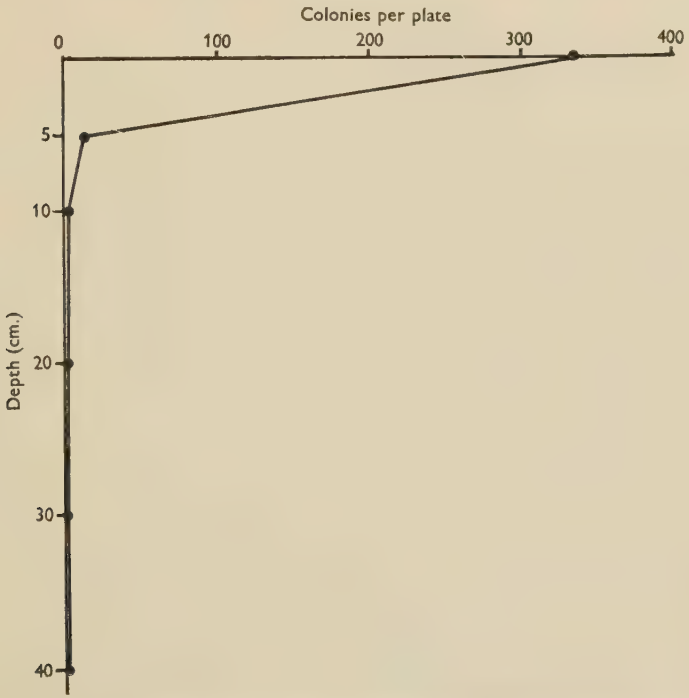

Fig. 4. Result of an experiment with a spore suspension of *Penicillium* using 200 c.c. of water.

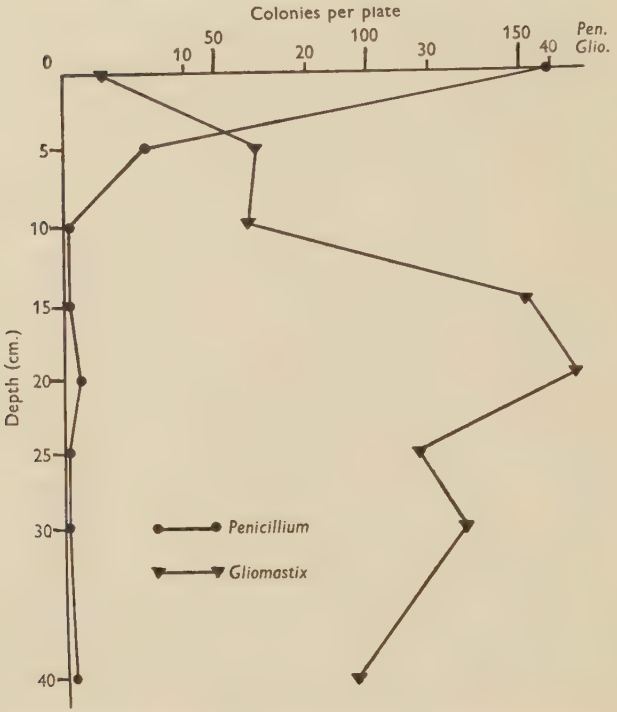

Fig. 5. Result of an experiment in which a mixed spore suspension of *Penicillium* and *Gliomastix* was used followed by 200 c.c. of water.

Discussion

It is clear from the experimental work that spores of *Zygorrhynchus* and *Gliomastix* wash readily through the sand column, whereas the *Penicillium* spores show very little movement. Both the former fungi have spores which have a mucilaginous coating to the wall. The sporangia of *Zygorrhynchus* break readily in water and the spores wet immediately. In *Gliomastix* the spores are 'slimed off' from the tip of the phialide and collect in a spherical mass. This mass disintegrates immediately on being transferred into water. Wakefield & Bisby (1941) have placed *Gliomastix* in the Gloiosporae, a group characterized by mucilaginous spore masses. Spore suspensions of these two fungi can be prepared readily as the spores are easily dispersed in water. Spores of *Penicillium*, on the other hand, showed practically no downward movement in sand. The spores of Penicillia are characteristically dry and powdery and the genus has accordingly been included in the *Xerosporae* by Wakefield & Bisby (1941). In preparing spore suspensions of this fungus the way the spores remain on the surface of the water and collect on the side of the tube is most striking. The older the culture from which the spores come the more noticeable is their resistance to wetting. Young immature spores wet more easily. In the column experiments with *Penicillium* it is easy to see the film of unwet spores remaining on the upper surface and resisting washing down. While it is not possible to be certain,

it seems highly probable that the small number of spores which do wash downwards are immature, with poorly developed spore coats.

The importance of the difference in behaviour of 'wet' and 'dry' spores is twofold. In the first place any conclusions drawn as to the vertical zonation of fungi based on the dilution-plate technique must be treated with reserve if the fungi concerned produce wettable spores. In the present series of investigations, for instance, the isolation of *Gliomastix* from all layers of the soil may mean that the fungus is active throughout the soil or that it is active and sporing in the upper layers and its spores have been washed down to the underlying soil. In fungi such as *Penicillium*, which produce dry spores, there is little likelihood of the spores being washed to lower levels.

There is also the ecological significance of the movement of fungal spores within the soil. Dispersal of the fungal spores may be either by air or by water. In the lower layers of the soil dispersal by water would seem to be the more efficient, and it is interesting to note that the majority of species isolated from the lower layers have wettable spores. On the soil surface, however, conditions are very different and dispersal by air currents probably plays a much more important role. A dry dusty spore mass would be readily dispersed by wind in contrast to a mass of spores held together by mucilage. An examination of the fungi isolated from the surface litter and the upper few centimetres of the soil shows a high proportion of species with dry spores.

Summary

Experiments were carried out to determine whether fungal spores can be washed downwards through a sandy soil. It was found that spores which have a mucilaginous coat wash downwards readily, while spores which have a waxy non-wetting coat remain on the surface.

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AQUATIC PREDACIOUS FUNGI

By MARY PEACH, B.Sc. *The Polytechnic, Regent Street, W.* 1

(With 11 Text-figures)

In the course of a general investigation of fungi appearing on maize-meal agar plates, after inoculation with plant debris from fresh-water ponds, ornamental and natural, I have identified a new species of *Dactylella*, and made the first records in Great Britain of *Dactylaria candida* (Nees) Saccardo, and *Acaulopage macrospora* Drechsler.

Dactylella reticulata sp.nov.

Mycelium hyalinum, septatum, hyphis saepe ramosis 4–5μ crassis; reticula ex laqueis 35–50μ diam. extus composita, quoque laqueo plerumque ex hypha separatim evolvente. Conidia in hyphis fertilibus, non ramosis ad altitudinem ca 250μ ferens; conidia hyalina, ellipsoidea, septata, 50–65μ longa, 20–25μ crassa, cellulis plerumque 5, interdum 5–6 quarum una media est maxima.

Vermiculos nematodeos capiens consumensque, hab. in foliis putridis arborum *Quercus petraea* (Matt.) Liebli., et *Fagus sylvatica* L. var. *cuprea* Loddiges, in cisterna aqua currente, in horto prope Harpenden Common, Herts.

A few small pieces of decaying leaves were placed on maize-meal agar plates, and after two weeks nematodes were seen trapped by networks produced by a hyphomycetous fungus. Under low magnification, the networks were clearly visible, being produced in fair profusion. The nematodes were captured apparently by adhesion, and an individual eelworm was often trapped by more than one loop of a network system (Fig. 1). The networks (Figs. 2–4) appeared to be produced by the anastomosis of loops, the loops arising separately from a main hypha, and lying in a variety of planes, so producing an efficient trapping system. The trapped eelworms struggled violently at first, moving more and more slowly as death came on. The cause of death was not determined, but the infection bulb present in dead specimens was always smaller in diameter than the host (Fig. 5). The infection bulbs measured 12–24μ in diameter, and the nematodes 15–30μ, in diameter. Assimilative hyphae, produced by the infection bulb, penetrated into the tissues of the eelworm, effecting digestion and absorption. Eventually the cuticle only remained.

Conidia were produced sparingly by the mycelium and usually in the region of a trapped eelworm, the conidiophores being simple, unbranched, septate, erect structures, each producing a single terminal conidium. Branching toward the base of a conidiophore occurred rather infrequently. At maturity the weight of the conidium often caused bending of the subtending hypha, and often the conidium touched the surface of the agar, and

germinated without being detached. The conidia were ellipsoidal to ovoid, and the proximal end usually narrowed abruptly. The number of cells in

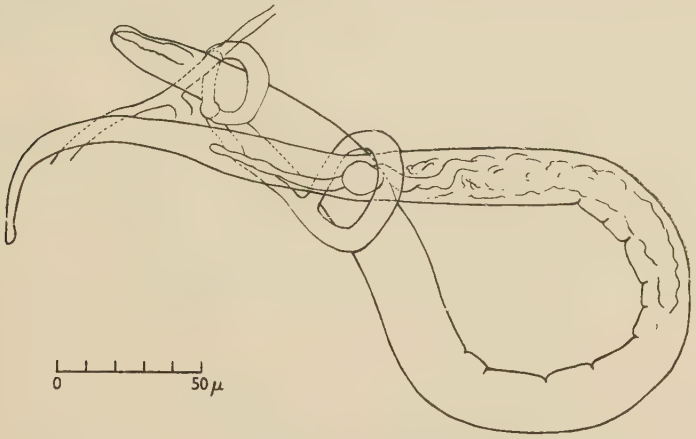
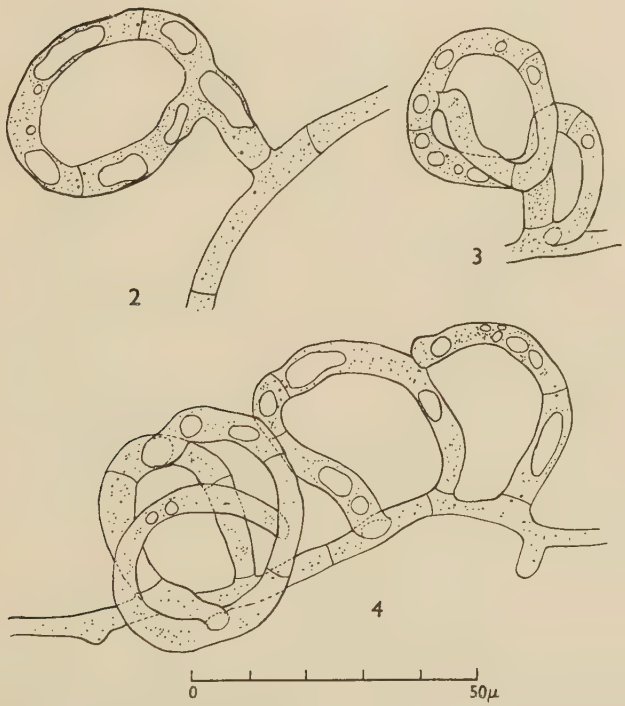


Fig. 1. *Dactylella reticulata.* Nematode captured by two loops.



Figs. 2–4. *Dactylella reticulata.* Networks at different stages of development.

each conidium was variable, but the majority were 5-celled (Fig. 6), and usually the central cell was the largest.

Germination resulted in formation of more than one germ tube, commonly produced from the ends of the conidium. Often a germ tube grew directly into a conidiophore. The fungus is easily cultured but no networks are produced in pure culture. If an eelworm culture is introduced, networks appear. No chlamydospores or resistant bodies have been observed.

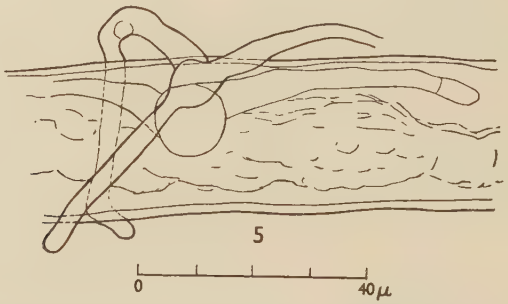


Fig. 5. *Dactylella reticulata.* Part of captured nematode showing infection bulb.

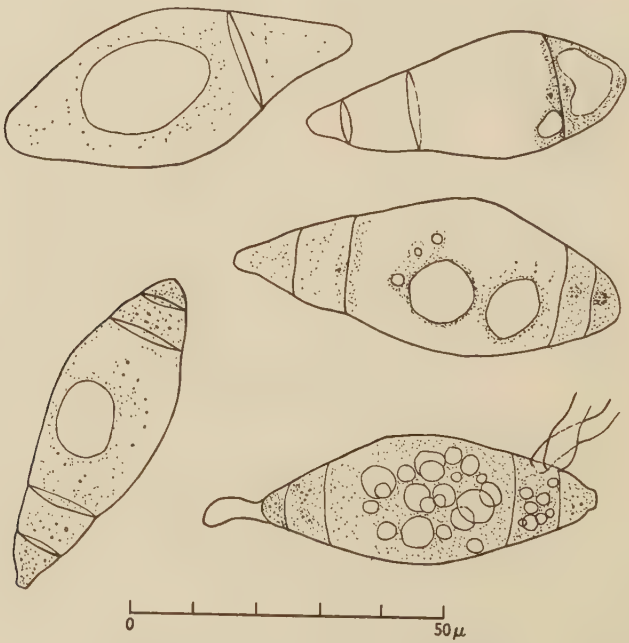


Fig. 6. *Dactylella reticulata.* Conidia.

This fungus does not resemble any species previously described.

Dactylella gephyropaga Drechsler (1937) produces anatomosing loops, but of a quadrilateral type, and conidia of a top-like form, and measuring 27–46μ in length and 16–21μ in diameter.

D. minuta Grove (1884), is described as a saprophyte, and the clavate conidia as being 6–8-septate and measuring 60–70 × 14–15μ.

D. minuta var. *fusiformis* Grove (1886) is also apparently saprophytic and has fusoid conidia measuring 60–75 × 7–9μ with 9–12 septa.

Clearly the fungus may be regarded as a new species, and is accordingly named *D. reticulata.*

Dactylaria candida (Nees) Saccardo

Leaf debris from the low-lying, long, ornamental pond in Chelsea Physic garden was used to inoculate maize-meal agar plates, and after a few weeks, a nematode trapping fungus was observed. In the early stages of growth, adhesive knobs on short stalks were most conspicuous and eelworms were being trapped by them. At a later stage, and in subsequent subcultures, the knobs were less frequent, and stalked rings were the main trapping devices (Fig. 7). An infection bulb producing assimilative hyphae with

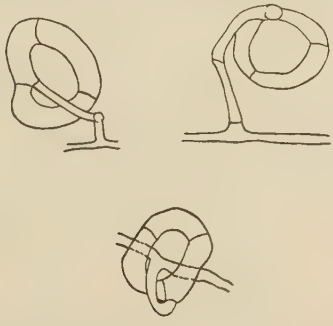


Fig. 7. *Dactylaria candida.* Stalked rings.

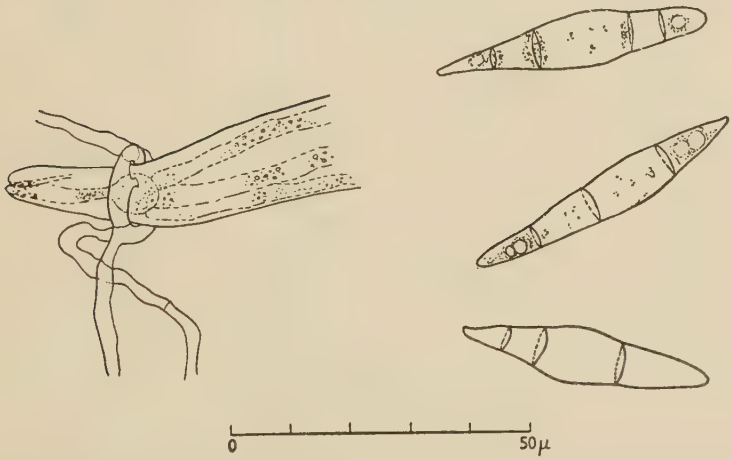


Fig. 8. *Dactylaria candida.* Captured eelworm showing infection bulb.

Fig. 9. *Dactylaria candida.* Conidia.

dilated ends was observed within the trapped eelworms (Fig. 8). Conidia were produced in great profusion, being borne in groups of threes and fours on unbranched erect, septate conidiophores. Each conidium was slender, septate, and the size range was 30–50μ in length, and 6–10μ diameter.

Conidia were commonly 5-celled, but those with four cells were not uncommon (Fig. 9). Frequently, the conidiophore developed from a region of spiral or coiled hyphae.

The fungus is easily cultured and produces an abundance of conidia. On germination commonly a germ tube was produced at each end, one terminating in a body resembling an adhesive knob, the other in a vegetative hypha, which produces a new mycelium.

In the form and nature of the adhesive knobs, and rings, and in the shape, dimensions and septation of conidia, the fungus agrees with *D. candida* (Nees) Saccardo, described in detail by Drechsler (1937).

Acaulopage macrospora Drechsler

From the same series of plates in which *Dactylaria candida* (Nees) Saccardo was recorded, a predacious phycomycete was observed and subsequently identified as *Acaulopage macrospora* Drechsler (1935).

The fungus produced a delicate, sparingly branched mycelium, and many haustoria were developed within amoebae. The haustoria were of the dichotomous type but of a simple pattern with a few forkings.

Conidia were produced in great profusion (Figs. 10, 11), each conidium

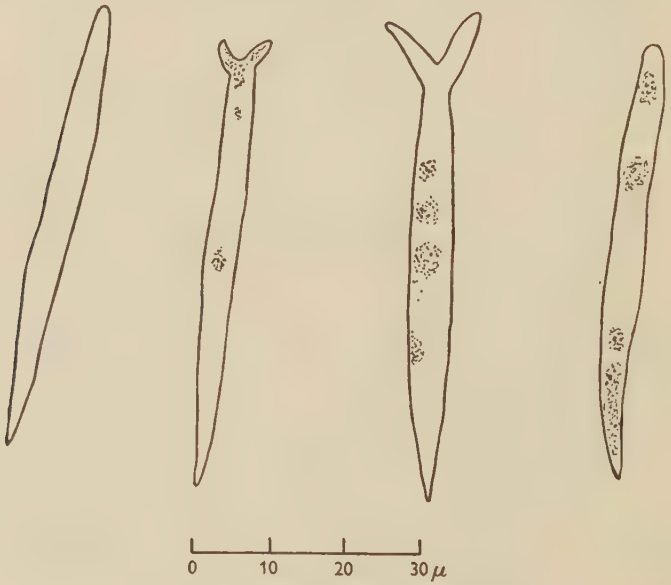


Fig. 10. *Acaulopage macrospora.* Conidia.

apparently developing as a simple, aerial, elongate body on a slender sterigma. At a later stage, the distal end forked, and just below the fork there was a slight constriction. The forked branches were not constant in length, and occasionally only one fork developed. At maturity, usually in both forks, the protoplasm was evacuated from the apical zone, and the terminal empty appendages so characteristic of species of *Acaulopage* were formed. Very occasionally one of the main forks underwent further forking.

The conidia were hyaline and presented an array of erect, rather hard-looking structures under low magnification. The cell wall is very definite, and in very old cultures, in which disintegration of vegetative hyphae has proceeded, the conidia are very conspicuous. Sexual reproductive bodies were not seen in this material.

This fungus is clearly *A. macrospora* Drechsler, but differs in several respects from his description. The haustoria were of simple dichotomous pattern, and most of the conidia forked, few remaining simple at maturity. The slight constriction below the point of forking is not mentioned by Drechsler.

This fungus was also identified from leaf debris obtained from a tank with running water in a garden near Harpenden Common, Herts.

I have obtained *A. dichotoma* Drechsler (1945) from *Elodea* collected from a pond at Moor Park, Herts, and again from the pond at Ken Wood, subsequent to my original record (Peach, 1948); *Acaulopage tetraceros* Drechsler (1935) has been isolated from the long, low, ornamental pond in Chelsea Physic Garden, London, and *Tetracladium setigerum* (Grove) Ingold (1942) was obtained from the *Elodea* inoculum from Moor Park.

Fig. 11. *Acaulopage macrospora.* Conidia.

I wish to thank Dr B. Barnes for his helpful encouragement during the course of the investigation, and Mr R. A. Gillanders for assistance in preparing the Latin diagnosis.

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DEFINITIONS OF SOME TERMS USED IN PLANT PATHOLOGY

(PREPARED BY THE SOCIETY'S PLANT PATHOLOGY COMMITTEE)

'When *I* use a word,' Humpty Dumpty said in rather a scornful tone, 'it means just what I choose it to mean—neither more nor less.'
'The question is,' said Alice, 'whether you *can* make words mean different things.'
'The question is,' said Humpty Dumpty, 'which is to be master—that's all.'
Through The Looking-Glass (CARROLL)

Confusion in the uses of technical terms employed in plant pathology was considered by the Plant Pathology Committee in 1942. No action was then taken, partly because a committee of the American Phytopathological Society had already begun to publish a list of definitions of such terms. In 1945 the matter was again raised. This time a subcommittee*, asked to consider the question, gave as its opinion that there was still scope for the Plant Pathology Committee to attempt the preparation of a list of its own, and in so doing to explore the matter more practically. The suggestion was accepted and the same subcommittee was entrusted with the task of bringing forward preliminary definitions which were subjected to detailed discussion and to amendment by the Plant Pathology Committee. Definitions approved by the Plant Pathology Committee were sent to the Plant Pests and Diseases Committee of the Association of Applied Biologists which had offered to help, and as a result of its criticisms some of the definitions were modified. It would be vain, however, to pretend that these definitions have had the unanimous approval of these bodies; some are acceptable only to a majority of the Plant Pathology Committee, while others are compromises.

We have not attempted to provide definitions for the use of other than plant pathologists. Some of the terms are well known also in applied entomology, biochemistry, or medicine; and it will be gratifying if in general our suggested usage accords with the main direction in a wider field.

As the American Committee has so wisely pointed out, all such definitions should be subject to continual scrutiny and revision. Our list is selective and open to many additions that will be obvious to readers: as well as appealing for comments and criticism we invite suggestions for further terms to be defined. These suggestions should be sent to Mr E. R. Wallace, 168 West Parade, Lincoln.

CARRIER — An organism harbouring a PARASITE without itself showing DISEASE

CONTROL — To prevent or retard the development of a disease

* The original members of the subcommittee were E. R. Wallace (convener), G. C. Ainsworth, H. E. Croxall and C. J. Hickman, with the then secretary of the Plant Pathology Committee, J. H. Western, *ex officio*. At the end of his term of office Dr Western was co-opted on to the subcommittee.

DISEASE	Harmful deviation from normal functioning of physiological processes
FUNGICIDE	Substance that kills fungus spores or mycelium
FUNGICIDAL	Able to kill fungus spores or mycelium
FUNGISTATIC	Able to prevent the growth or development of fungus spores without killing them
PROTECTANT	A substance that protects an organism against infection by a PATHOGEN
PROTECTIVE FUNGICIDE	A FUNGICIDE used as a PROTECTANT
ERADICANT FUNGICIDE	(1) A FUNGICIDE applied to a substratum in which the fungus is already present (2) A FUNGICIDE used in disease control after infection has been established
HABITAT	Natural place of occurrence of an organism
HOST	An organism harbouring a PARASITE
POTENTIAL HOST	An organism capable of harbouring a particular PARASITE
HYPERSENSITIVE	Giving violent local reaction to attack by a PATHOGEN, the prompt death of tissue round the points of entry preventing further spread of infection
IMMUNE	Exempt from infection
INFECT	(Of a micro-organism or virus). To enter and establish a PATHOGENIC relationship with an organism; to enter and persist in a CARRIER
INFECTIVE	Able to infect. See Viruliferous
INOCULATE	(1) To introduce a micro-organism or virus into an organism (2) To introduce a micro-organism or virus into a substratum
INTOLERANT*	Killed rapidly when invaded by a given PATHOGEN
PARASITE	Organism or virus existing in intimate association with a living organism from which it derives an essential part of the material for its existence while conferring no benefit in return
PATHOGEN	A PARASITE able to cause DISEASE in a particular HOST or range of HOSTS
PATHOGENIC	Possessing the attributes of a PATHOGEN.
RESISTANT	Possessing qualities that hinder the development of a given DISEASE
SAPROPHYTE	An organism using dead organic material as food
SENSITIVE	Reacting with severe symptoms to the attack of a given PATHOGEN
SUBSTRATUM	The material on or in which a SAPROPHYTE is living
SUSCEPTIBLE	NON-IMMUNE
TOLERANT	Able to endure infection by a particular PATHOGEN, without showing severe DISEASE
VECTOR	An animal able to transmit a PATHOGEN
VIRULIFEROUS†	(Of a VECTOR) Containing a VIRUS and able to transmit it.

On orismology

Orismology is the science of defining technical terms; in origin it implies setting bounds to them. But because the situation to be labelled is always growing and changing, boundaries are ephemeral and a good definition may be expected to survive longer if set up rather as a signpost to point

* The use of this term is not recommended. It should not be used as a synonym of SENSITIVE.

† The use of VIRULENT in this sense is deprecated.

clearly in one direction. A robust measure of ignorance about the difficulties likely to be involved is prerequisite even for the less ambitious task. No one who could hold them all in view at once would ever venture into a field where one man's common sense is another man's pedantry, haunted by influences that are powerful because unrecognized. As often as not, the accepted facts in a science, marshalled under the prevailing theories or hypotheses, are temporarily fitted into fundamentally conflicting philosophies, especially powerful when the holders are unconscious of them, and these tacit assumptions colour the interpretation of technical words in use at the time. Those who have read any popular work on semantics will probably have come across the notion of a word as a label attached afresh on each occasion to a meaning unique and arbitrary, depending on the total situation at the time and on the history of the user. (In the same way the meaning for each listener or reader will be unique and different.) Since we are dealing with a few words in a highly specialized field of work, our chances of mutual understanding should be much greater than the above would suggest, but it should be remembered that these different personal philosophies are inescapable. It is an advantage that technical words have the minimum of emotional content, but the same is not always true of the auxiliaries that we may seek to utilize in definitions. Phrases like 'vital processes' or 'vital activity' have a flavour linking them with 'vitalistic' theories and are therefore not suitable.

Attempted classification of technical terms is not likely to help much, but it may be useful to remember that their histories are very diverse. Words made up *ad hoc* out of ancient Greek fragments and applied to plant or animal parts will tend to persist even if the function of the part was misunderstood at the time. The same is true, much less justifiably, of words of the same type applied to biological processes of which the 'destinations' were likewise misunderstood. There are also words forming part of the apparatus of any new hypothesis, necessary before it can become articulate; if this is afterwards shown to be false or unnecessary, these ought properly to disappear. Words defining the relationships between different organisms live a hazardous life in the zone where theory is applied to process. In a younger science, both 'Greek' words and common words may be borrowed through some older science, or the latter kind may be adopted direct from everyday speech, with narrowed meaning. In the latter instance, the restriction is usually well understood, but with the other kind of borrowing, the inevitable new change of meaning may be slurred over at first. When such jargon words in 'different' usage survive alongside one another in fields of study that come to overlap, such as medicine (animal pathology) and plant pathology, the difficulties are such that the beginner cannot distinguish between 'loose' and 'different' usages, and it is all the more necessary that he should have some explicit guidance.

Aesthetic considerations are not usually openly acknowledged, but they play a part in the success or failure of new technical terms and those who cannot appreciate this should not themselves coin new ones. On this side of the Atlantic 'klendusity' and 'suscept' were each thrown out without a hearing, because they are the very opposite of comfortable words. Yet there are concepts which urgently need the equivalent labels in some

acceptable forms. Obviously the parallel '. . . as PASASITE is to HOST, so is PATHOGEN to . . . ' requires a missing term. If we were to jargonize VICTIM, as might be done, at once new difficulties would arise.

Anyone who has read so far and needs refreshment would do well to turn to the preamble to the Report on Technical Words of the American Phytopathological Society (1940) which is both lucid and learned. It makes the very useful working distinction between 'different' usage and 'loose' usage that has been implied above, and goes on to say 'the loose use of such terms as *disease* and *epidemic* causes little confusion or misunderstanding, whereas continued misuse of such words as *immunity*, *resistance*, *tolerance* . . . tends definitely to confuse or mislead readers.' It also contains the following '...It may be well to emphasize in advance, however, that while we do not regard our definitions as final, or indeed, in some cases, as very satisfactory, we believe that even an imperfect definition may be well worth study.'

In essaying our own list of definitions we had the American one before us and thereby avoided a host of preliminary difficulties, but on one aspect of procedure we took up a different point of view and this had far-reaching effect. The Report stated '...We also have tried to make each definition a unit; that is, understandable without references to another definition, unless it referred to one immediately preceding.' On the other hand, the present committee tried to make the definitions in certain groups depend on one another. Absolute formal consistency is only possible between mathematical terms, so this may have been an unwise thing to attempt, but in discussing such a chain of definitions serious hidden inconsistencies may be turned up that otherwise might have been glossed over. No doubt there are others surviving.

It is now appropriate to consider some of these relationships in detail.

Any definition of DISEASE was bound to follow the negative pattern of the word itself and mirror the idea of a falling away from some human standard of excellence, itself otherwise undefined. The American definition contained the phrase '...of sufficient duration or intensity...' implying that there are physiological disturbances of a slight or temporary nature that it is not useful to classify as 'disease'. After considering this view, the Plant Pathology Committee rejected it. No short definition will bear much logical scrutiny; the one put forward can best be criticized on whether or not it is a useful point of departure for other nearly-related definitions, such as that of CARRIER. This last definition also contains PATHOGEN, which in turn was made dependent on PARASITE. Here we found ourselves in complete agreement with the American Committee about the difficulty as to whether a virus may be considered to be a living organism, and we concurred with their use of 'organism or virus' in the key definition. Whatever the status of viruses, their dependence on living tissues for increase is not in dispute at present, and a mere inclusion of them under PARASITE should not force anyone to consider them as organisms.

The definition of HOST is to be read in conjunction with that of PARASITE. Usage attaches both HOST and CARRIER to lists of species and varieties, the members of which are capable of harbouring the parasite in their living tissues. The individuals are POTENTIAL HOSTS (including POTENTIAL CARRIERS), but there seemed no need to cavil at the phrase HOST LIST, when

this was so well understood. It being commonly agreed, however, that a plant harbouring a saprophyte or saprophytic stage in its dead parts, detached or otherwise, was not to be listed as a host of that organism, discussion of SUBSTRATUM and HABITAT was immediately forthcoming. The peculiar use of 'SUBSTRATE' by workers dealing with enzymes was noted, and it was felt that such a clear-cut usage need not be trespassed upon by 'translating' SUBSTRATUM into the English shape for any other sense. SUBSTRATUM itself, now quite comfortably adopted, is the better form to retain all general implications from which we can derive a narrowed biological use—'a material on or in which a saprophyte is living'. This throws the emphasis of HABITAT, in lists, on the natural *place* of occurrence of an organism as against the material support. For the ecologist it is a much more comprehensive term, implying the totality of the natural environment, but it is an open question whether these two different uses clash at all seriously. (The adjective 'natural' holds difficulties for some who are not satisfied with the notion that, for instance, the natural place of occurrence of a cultivated wheat is the place where it has been sown by man—owing perhaps to the too common belief that man is unnatural! Anyone who clings too rigidly to an antithesis between 'natural' and 'artificial' should be asked whether the strains, say of a rust, found only on such cultivated wheats are not in fact already in artificial culture.)

Alongside PARASITE, it was considered necessary to define SAPROPHYTE, and a definition—'An organism using dead material as food and commonly causing its decay'—had the last phrase pruned on the ground that ascription of such a complex affair as decay to any single organism as causal agent was altogether too simplified a conception. We considered the proposition: are all NON-PARASITES SAPROPHYTES? If so the definition had better have been amended to read 'non-living organic material'. 'Organic' hides two meanings; after non-living it might only imply carbon compound, so that the instance of an organism in culture receiving its carbohydrate out of the bottle would be covered. This brings us a long way from σαπρος 'rotten', and the antithesis is not particularly valuable, so the definition was left. (-PHYTE means plant and SAPROBIOTIC, SAPROZOOTIC exist but give rise to ugly nouns, while dictionaries already acknowledge the widening of SAPROPHYTE to cover organisms that are not plants.)

VECTOR was introduced somewhat arbitrarily because of the verbal connexion with CARRIER (which has some history of confusion behind it). An original definition with the phrase '...an organism capable of movement' had this replaced by 'animal'. (It was clear that whatever the status of the dodders as channels of transmission for viruses, it was not desirable to describe them as vectors).

To use INFECT of an agent in the sense of to bring about infection with an organism, did not commend itself, especially when considered alongside INOCULATE. The American definition of INFECT—'to invade an organism'—with the explanatory note upholding precise usage, cannot be bettered for clarity, especially if the dictionary definitions of 'invade', which correspond with its military use, are borne in mind. But sometimes, perhaps wrongly, 'invade' is thought of as simply equivalent to 'ingress', 'enter', and against such possible misinterpretation, we wished to stress the establishment of the

disease-causing relationship and also the persistence in a carrier, which are bound up with our convenient usage of INFECT, INFECTION and INFECTED. The last with its 'present' sense is a term that workers in the field of plant hygiene need for labelling all living host reservoirs of a particular pathogen, even during the stages or periods when transmission is not active or feasible (i.e. when they are not necessarily INFECTIVE). A plant may suffer some malformation during the progress of an infectious disease; the pathogen may afterwards die out. By definition the plant is still DISEASED (and indeed its physiological perfection is presumably still impaired) but it is no longer INFECTED. We have no single word to mean 'having been, but no longer infected', were such a state to be artificially contrived, neither 'recovered' nor 'cured' is necessarily correct, and DISINFECTED is perhaps the most deplorable example we have of a needed meaning completely spoiled by wrong usage.

Closely connected with INFECT, at least in looser usage, is INOCULATE. Here the American comment was—'It does not seem worthwhile to discourage the common and well-established usage with reference to inorganic substrata, often criticized on the grounds that one cannot inoculate that which cannot become diseased'. In agreement with this, two definitions have been offered. Neither the American ones nor these cover the activity of a research worker introducing material which he only hopes or believes may contain the micro-organism or virus, but this does not seem a very serious defect. It will be noted that more than once 'micro-organism' has been used, which is itself not a well-defined term. There are many pathogenic fungi that are not microfungi in the mycologist's sense, for instance. On the other hand, the use of 'organism' alone led to such exchanges as the following, found at the foot of an otherwise dignified sheet: '...Could you not speak of INOCULATING a swimming-bath by putting twenty people (organisms) in?...More like CONTAMINATING it!'

Over and over again we have found it more profitable to consider descriptive terms from the point of view of an experimental worker and his actions and observations rather than from that of any system of plant pathology. Unfortunately, the group of terms including IMMUNE, RESISTANT, SUSCEPTIBLE, TOLERANT, SENSITIVE offers difficulties that are not lessened by this approach. Discussion soon exposes differences in attitude of mind about the interpretation of agreed facts of observation. Thus, if under various conditions, the organism (or virus!) P is brought into contact with the plant varieties X, Y, Z, under some conditions infection is successfully brought about, resulting in disease, and P is therefore a pathogen. But under all conditions we are able to try, this relationship fails to be set up with the varieties A, B, C, etc. We therefore list X, Y, Z, as SUSCEPTIBLE and A, B, C, as IMMUNE, which is convenient. But the word IMMUNITY is a description relating to $A+P$, $B+P$, etc.: it is not an attribute of A and B solely. Similarly, PATHOGENICITY might be used as a description about $X+P$, $Y+P$, etc., and not merely be thought of as an attribute of P. In practice we certainly often wish to list varieties as 'immune', or 'susceptible' long before the exact nature of these relationships can be explored, when it is yet premature to credit the plant alone with 'immunity' or any other attribute.

More than one participant demanded CONTROL, and a definition was

forthcoming without serious disagreement. One doubt assails the cautious —ought we to speak of 'controlling' a disease by those measures (such as the use of immune varieties) that imply evasion rather than positive mastery of the trouble?—it is a question perhaps best left to the individual writer.

Next some recognition of increasing standardization among fungicide terms (American Phytopathological Society, 1943) seemed to be due, and definitions of FUNGICIDE, FUNGICIDAL, FUNGISTATIC, PROTECTANT and PROTECTIVE and ERADICANT FUNGICIDE were put forward without any real disagreement on meanings. There is no noun corresponding to FUNGISTATIC: 'FUNGISTAT', inserted merely to provoke discussion, was deprecated, but it is surely no queerer on the English tongue than RHEOSTAT.

Since the general plant pathologist must needs give increasing attention to virus diseases, the intrusion of such a term as VIRULIFEROUS can scarcely be avoided. It seems elementary to point out that the function of this term cannot be transferred to VIRULENT, however painstaking the experimenter may be. Those who do not like VIRULIFEROUS as a word should either say 'loaded with active virus' (involving an assumption) or amend their sentences to avoid it altogether.

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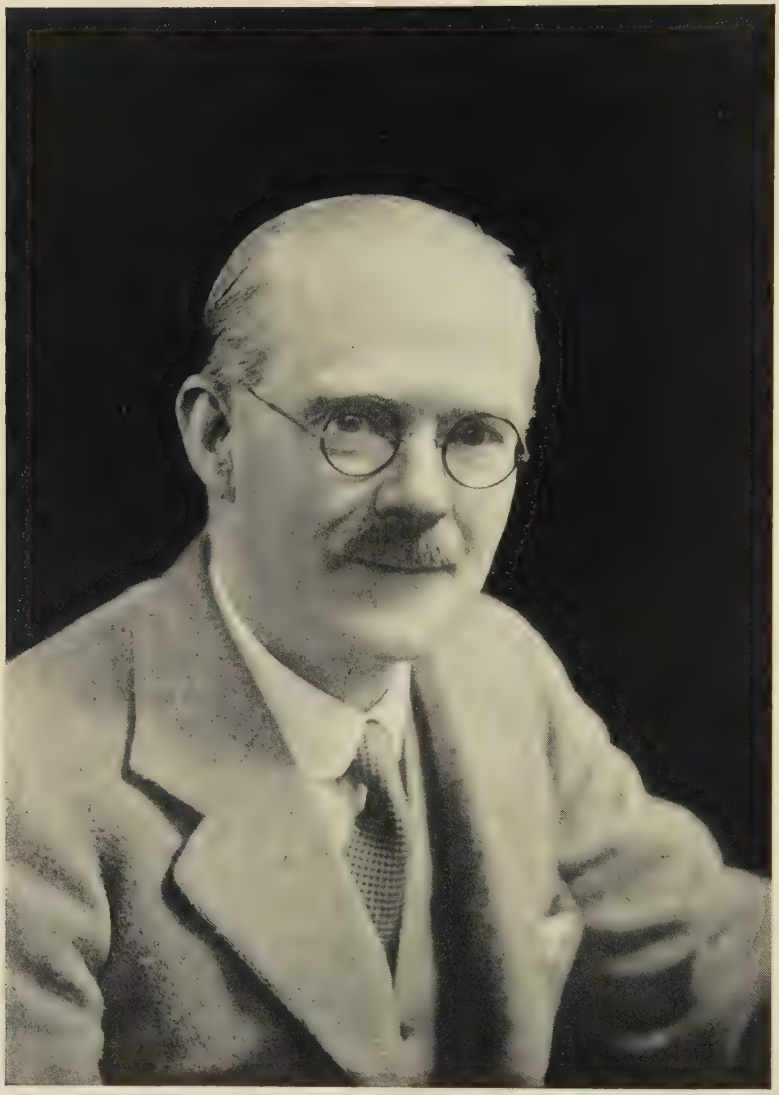

Dr G. H. PETHYBRIDGE

OBITUARY NOTICES

Dr George Herbert Pethybridge, O.B.E., B.Sc., Ph.D., 1871–1948

In any society in any year, no matter how its ranks may be made replete through the recruitment of the enthusiasm and vigour of youth, there will occur gaps. Some will be hardly perceptible and will close quickly—almost unobserved—but there will be others which will yawn wide open, causing a breach seemingly incapable of repair. Such a gap in the ranks of the British Mycological Society and of economic biology has been caused through the death of George Herbert Pethybridge.

Geo. H. Pethybridge—to refer to him in terms of his own signature, and to which of us who knew him well will this reference not bring to mind the bold and deliberate stroke of that pen which accomplished so much—was born at Bodmin in Cornwall on 1 October 1871. Schooled in Bodmin and Launceston he decided against the family profession of banking and matriculated through Owens College, Manchester. He commenced his scientific studies at the University College of Wales, Aberystwyth, and after obtaining an honours degree became, for a short time, a schoolmaster. Later he went to Germany and studied at Göttingen under Berthold, where he worked in the field of plant physiology and graduated as Ph.D. On his return from Germany he accepted an appointment at the Royal College of Science, Dublin, where for some twenty years he was destined to play a leading role in the development of economic biology as fostered and administered by the Department of Agriculture and Technical Instruction for Ireland after its foundation in 1900. In 1908 he was appointed Economic Botanist and Head of the Seeds and Plant Disease Division of the Department, a post which he occupied until 1923 when he resigned and his career in Ireland came to a close.

It was from 1908 to 1923 that Pethybridge achieved what was probably his best work as an experimental scientist and for which he will be most remembered. The outstanding importance of the potato crop in Ireland, emphasized by the tragedy of the famine, made it only natural that priority should be given to the study of the diseases of the potato, and it was to this subject that he gave his greatest attention. He approached the problem with a precision and thoroughness which was to become so characteristic of all his work, and deciding rightly that the line of attack must be made through experimental plant pathology, he insisted upon the establishment of a field laboratory in a district where potatoes were grown intensively and where the incidence of disease was considerable. Such a district is Galway in the west of Ireland and it was there, at Clifden, that his field laboratory was situated. In each of the ten years, 1910-1919, a report was published in the *Journal of the Department of Agriculture and Technical Instruction for Ireland* dealing with the work which had been done and the results obtained. Entitled 'Investigations on Potato Diseases' these reports, which are well illustrated with excellent photographs, are written in that clear and lucid style which Pethybridge was to make his own and which was so successful in reducing highly technical matter so that it

may be readily followed and easily understood. The more scientific aspects of his work appeared, again so clearly described and well illustrated, in a series of papers published in the *Scientific Proceedings of the Royal Dublin Society* and of the *Royal Irish Academy*. In the latter years he was joined by Paul Murphy who was eventually to succeed him and contribute largely to the study of potato virus diseases. The high lights of this work dealt with the problem of the overwintering of *Phytophthora infestans* and the search for oospores; the investigation of pink rot and its cause *P. erythroseptica*; the discovery of amphigynous antheridia in the genus *Phytophthora*; *Verticillium* wilt, dry rot, and last but not least, the investigation of the control of potato blight by spraying.

The war of 1914–18 brought about a large temporary increase in the acreage of fibre flax and very soon, under conditions of more intensive cultivation and through the difficulty of obtaining adequate supplies of fresh seed, the incidence of disease in the crop assumed significant proportions. Thus, from 1917 onwards Pethybridge took flax under his wing and again, insisting upon the necessity for study in the field, he succeeded in establishing a field laboratory for the work, this time at Bog Hill, Coleraine in Co. Londonderry, the centre of a flax district. Here, ably assisted by H. A. Lafferty (plant pathology) and J. G. Rhynehart (entomology), a great deal of pioneer work was carried out and three annual reports following upon the same lines as those for potato diseases appeared in the Department's *Journal* from 1920 to 1922. Most outstanding was the work dealing with the life histories of *Colletotrichum linicola*, the cause of seedling blight in flax, and *Polyspora lini*, the cause of stem break and browning. It has been the writer's privilege to study and repeat much of the work of Pethybridge and his colleagues, particularly in the case of flax, and apart from the ease with which the work can be followed, the accuracy of statement and description is unique.

Besides his work on the diseases of the potato and flax, Pethybridge's investigations in Ireland covered a wide field as is instanced not only by his occasional study of other crop diseases but by his census catalogue of Irish fungi prepared in collaboration with J. Adams, and a study of the vegetation of the district south of Dublin carried out in partnership with R. Ll. Praeger. He was also responsible for the supervision of the Dublin Seed Testing Station which was the first established in the British Isles.

Not much is heard outside Ireland of Pethybridge as a teacher, but that he also excelled in the handling of his classwork in the Royal College of Science soon becomes evident in discussion with those who were once his students. Many of them were outlived by their master but those who still survive speak with great respect and sometimes almost with awe of their course of instruction under Dr Pethybridge. There was obviously no room for backsliders at the bench of that laboratory and few could have come away without a sound training in the principles of plant pathology. Only the other day, in the course of a somewhat inconclusive discussion at a Conference in Northern Ireland, an elderly gentleman who had been his student arose to announce with emphasis: 'Didn't Pethybridge say "Many diseases are due to disharmony with environment?"' There were no further comments.

Pethybridge's outstanding work in Ireland was crowned in 1921 when he was awarded the Boyle Medal by the Royal Dublin Society (*Sci. Proc. R. Dublin Soc.* **16** (N.S.), 226, 1921).

In 1923 he left Ireland and accepted the post of Mycologist to the Ministry of Agriculture and Fisheries at the Plant Pathology Laboratory, Harpenden; later he was appointed Assistant Director of the Laboratory. Here again for thirteen years he was to give of his best to the science to which he devoted his life. Not so much on the experimental side this time but rather in advising on technical matters and in encouraging and co-ordinating the work of the Advisory Plant Pathology Service in England and Wales. The quality of his writing and his reports retained their high standard of accuracy and readability and, as in Ireland, he proved himself a leader and master of his craft.

Pethybridge joined the British Mycological Society in 1919 and was honoured by election to the office of President in 1926, the title of his address being 'Mycology and Plant Pathology'. He was an active member of the Royal Irish Academy, the Royal Dublin Society, the Royal Horticultural Society, the Linnean Society and the Association of Applied Biologists.

Retiring from the service of the Ministry of Agriculture and Fisheries in 1936 he was made O.B.E. in 1937 in recognition of his outstanding work. For some ten years subsequent to his retirement he served as Assistant Editor of the *Journal of Pomology and Horticultural Science*, an appointment for which he was admirably fitted, and vol. **23** of the *Journal* was dedicated to him in acknowledgement of his service.

Although officially retired and returned to his native heath at Bodmin, Pethybridge kept up his correspondence and interest and was always ready with advice and assistance when required. His letters still remained full of that pointed good humour which permeated the whole of his active life and seldom was the discussion of technical matters not accompanied by some rare tit-bit which revealed a piquant and tantalizing personality underlying the plant pathologist. In February 1948 the familiar handwriting and signature remained as clear and bold as ever; by May that firm hand showed signs of losing its cunning and on 23 May 1948 he died. A valiant Cornishman had passed on but there still remains a treasured memory and a sterling example. A.E.M.

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Miss GULIELMA LISTER

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Miss Gulielma Lister

With the passing of Miss G. Lister, Mycology has lost yet another of its outstanding workers, and the Society an old and much-loved friend.

Gulielma Lister was born on 28 October 1860, in the family house at Leytonstone, where she died on 18 May 1949. She came of a Quaker family distinguished for its learning and artistic ability. Her grandfather, J. J. Lister, F.R.S., was an eminent physicist and microscopist, and one of her uncles was Lord Lister, the famous surgeon. Her father, Arthur Lister, F.R.S., by trade a wine-merchant, was a keen all-round naturalist and a good draughtsman who eventually was attracted to the study of the Mycetozoa, and in 1894 published a monograph which became the standard work on the group. Her mother was a good amateur artist, who had studied under David Cox, so that the scientific and artistic abilities of the children were derived from both sides.

Gulielma, one of seven children, was educated at home, except for one year at Bedford College, and became the constant companion of her father in his studies of natural history. In the preface to his work on the Mycetozoa Mr Lister stated that throughout his studies and in the preparation of the drawings he had been assisted by his daughter. After his death in 1908 Miss Lister was responsible for the second (1911) and third (1926) editions of this standard monograph, in which through her generosity many of the illustrations were reproduced in colour. She became the world authority on the group and received material from all quarters of the globe.

Miss Lister joined the British Mycological Society in 1903 as one of the first 100 Foundation Members, and throughout her life did much to help the Society. Until a few years before the last war she was a regular attendant at forays, and with a few enthusiastic helpers always staged a good show of her beloved 'creepies'. She was twice President, in 1912 and 1932, and in 1924 was made an Honorary Member in recognition of her services to Science and to the Society. From the time the Council was established Miss Lister's quiet influence was of the greatest value in the conduct of the affairs of the Society.

She was closely associated with the activities of the Essex Field Club and, until just before the war, was a regular attendant at its fungus forays. She was also greatly interested in the School Nature Study Union, and from 1918 to 1934 guided its Executive, first as Vice-Chairman and then as Chairman. She was elected to the Linnean Society in 1904 when fellowship was opened to women, and was twice a member of the Council, from 1915 to 1917 and again from 1927 to 1931. From 1929 to 1931 she was a Vice-President.

In addition to being the authority on the Mycetozoa Miss Lister, like her father, was a good general naturalist. With Miss Hibbert-Ware she studied especially bird-life, and on fungus forays usually carried with her a small telescope with which to observe any interesting birds. Her artistic ability was manifested not only in the illustrations to the Mycetozoa Monograph but also in the figures for the *Handbook of the Coniferae* by Dallimore and Jackson, and the beautiful coloured plates in F. J. Hanbury's *Illustrated Monograph of the British Hieracia.*

Miss Lister's work was characterized by painstaking, accurate methods of observation and recording. She possessed in the highest degree the qualifications of the true scientist, an unbiased mind to search after truth, and readiness both to learn and to teach. To many a younger student her occasional quiet criticism and sound advice must have been a stimulus. When she was no longer able to take an active part in the affairs of the Society she was greatly missed. We pay tribute to a long and useful life.

E.M.W.

Professor Sir Rowland H. Biffen

Sir Rowland Biffen, President of the British Mycological Society in 1905 and again in 1930, died at his home in Cambridge on 12 July 1949, aged 75. Born at Cheltenham, he received his early education at the local grammar school. From his father he acquired an abiding interest in field botany, learning his plants as a boy by long excursions into the Cotswold country. He entered Emmanuel College, Cambridge, with an exhibition in 1893, was elected a scholar in 1894, and graduated in 1896 with first classes in both parts of the Natural Sciences Tripos. Thereupon he was appointed Frank Smart student in botany at Gonville and Caius College and devoted himself to research in mycology, although this was interrupted by a visit with Mr E. Howard to Mexico, Brazil, Trinidad and Mexico in order to study methods of preparing rubber from latex-bearing trees. He spent a considerable time in Amazonia especially on the river Tocantins, and the north-eastern part of Brazil. He always retained a vivid recollection of these wanderings. As a result of his investigations he invented a centrifugal process for the coagulation of raw latex.

In 1898 he was awarded a research studentship at Emmanuel College and was also appointed university demonstrator in botany by Prof. Marshall Ward. He made contact with Dr C. B. Plowright of King's Lynn and used to hunt for fungi with him in the woods around Brandon. In the early days of the British Mycological Society, Biffen often attended the autumn forays. During the period when mycology was his principal

PROFESSOR SIR ROWLAND H. BIFFEN

interest he published the following papers: 'On the biology of *Bulgaria polymorpha*' (*Ann. Bot., Lond.*, **17**, 119, 1901), 'On the biology of *Collybia velutipes*' (*J. linn. Soc.* (*Bot.*), **34**, 147, 1899), 'A fat-destroying fungus' (*Ann. Bot., Lond.*, **13**, 363, 1899), and 'On some facts in the life-history of *Acrospeira mirabilis*' (*Trans. Brit. mycol. Soc.* **2**, 17, 1902). Even after he became chiefly concerned with plant breeding he retained his love for mycology, and stimulated Miss D. E. Marryat in her investigations on the histology of infection by *Puccinia glumarum* (*J. Agric. Sci.* **2**, 129, 1907), Miss E. Dale in her studies on soil fungi (*Ann. mycol., Berl.*, **10**, 452, 1912 and **12**, 33, 1914), and G. T. Spinks on 'Factors affecting susceptibility to disease in plants' (*J. Agric. Sci.* **5**, 231, 1913). The present writer gratefully acknowledges Biffen's assistance in mycology after Marshall Ward died in 1906. As Biffen became more and more immersed in cereal breeding he took special cognizance of the rust fungi, and his presidential address to the Society in 1930 on 'The cereal rusts and their control' (*Trans. Brit. mycol. Soc.* **16**, 19, 1931) was a masterly survey of knowledge at that date.

In 1899 Biffen was appointed lecturer in botany in the rapidly developing Cambridge department of agriculture, and in the course of a few years, together with T. B. Wood, professor of agriculture, raised it to a pinnacle of fame. For a long period he lectured and demonstrated single-handed to the students on all aspects of agricultural botany while pursuing his own researches on cereal breeding in the most ardent manner. In 1900 came the re-discovery of Mendel's classical paper on the inheritance of certain plant characters, and Biffen at once saw its immense importance in relation to cereal breeding. He immediately set to work at the old university farm at Impington to test the validity of Mendel's laws in wheat and barley, and soon demonstrated their applicability to these crops. His papers entitled 'Studies in the inheritance of disease-resistance' (*J. Agric. Sci.* **2**, 109, 1907 and **4**, 421, 1912), in which he showed that susceptibility and resistance of wheat varieties to yellow rust are transmitted in Mendelian fashion, were a landmark in the history of plant breeding, and stimulated investigations all over the world on the control of diseases of economic plants by breeding resistant varieties. This is not the place to discuss Biffen's other contributions to plant genetics, but a word must be said about his insight into economic problems. In trying to improve still further the yield of English wheat he realized that the introduction of resistance to yellow rust would probably be efficacious. So he hybridized one of the standard English varieties, Square-head's Master (susceptible), with an exotic variety, Ghirka (very resistant); one of the segregates of this cross is the well-known variety, Little Joss. The origin of this peculiar name is not without interest. One day the late Sir Rider Haggard, much interested in agriculture, was viewing Biffen's new wheats at Impington. The visitor saw one outstanding variety and asked for the name of this 'little josser'. Biffen replied that it had not yet been named but that he would henceforth call it 'Little Joss'! The other major problem confronting Biffen in wheat breeding was to produce for this country a heavy-cropping, short-strawed variety, the 'strength' of whose grain would make a well-shaped loaf like the Canadian wheats graded as Manitoba Hard. Biffen solved this problem by breeding the variety Yeoman, one of whose parents was the Canadian variety Red Fife.

In 1908 Biffen was appointed professor of agricultural botany in the University and was elected a professorial fellow of St Catharine's College. In 1911 much better facilities for plant breeding on the new university farm on the Huntingdon Road were provided by the Development Fund through the Ministry of Agriculture, and Biffen became the first director of this Plant Breeding Institute, which was attached to the School of Agriculture. He also had much to do with the foundation of the National Institute of Agricultural Botany in 1919, which, among other activities, tests on a commercial scale the new varieties produced at the Plant Breeding Institute. In 1926–7 Biffen visited Kenya in order to advise the government on wheat breeding and cultivation there, and found this country most attractive. For many years he was consulting botanist to the Royal Agricultural Society.

Biffen retired from his professorship in 1931 but continued as director of the Plant Breeding Institute until 1936. On relinquishing his official duties he devoted himself, with the help of his wife, to his large and beautiful garden, continuing to hybridize delphiniums, gladioli, auriculas and other plants, and taking special delight in growing choice alpines. With his outstanding skill as a plant breeder he had already produced new varieties of sweet peas, garden peas and strawberries in earlier years. Just before he died he completed the manuscript of a book on the garden Auricula, a plant of which he had made a life-time study.

Biffen was elected a fellow of the Royal Society in 1914 and received its Darwin medal in 1920. He was knighted in 1925. For many years he was an honorary fellow of Emmanuel College and was an honorary D.Sc. of Reading University.

Biffen was extremely versatile, for, in addition to his extraordinary knowledge of plant life and agriculture, he was a highly skilled engineer and an extremely good water-colour artist. He was one of the most reserved of men, but when one got to know him well his insight, sound judgement and wide interests in life were most impressive. No one could be a more attractive companion either in a walk through wild country or while examining crops on a farm. He took a special interest in Breckland and for many years was the chairman of the Cambridge Breckland Research Committee. He married Mary Hemus, of Upton-on-Severn in 1899. She was an expert gardener and his constant helpmate in all his undertakings until she died in 1947; there were no children of the marriage.

F. T. BROOKS

PROCEEDINGS, 1947

THE EXETER FORAY

By G. SMITH, *Foray Secretary*

13–19 September 1947

Between 50 and 60 members attended the annual Autumn Foray at Exeter. Accommodation was provided in two of the halls of residence of the University, Duryard House and Thomas Hall, with headquarters and workroom at the latter. Although the west had had appreciably more rain than the London area, the ground was much too dry to please the agaric hunters. One gathered the impression, especially when heavy rain fell during the week, that another visit, in about two weeks' time, would be more profitable. As in 1946, when the condition of the ground was just the opposite, the most successful collecting was of microfungi.

On Sunday most members explored the College grounds, only a few of the more energetic going farther afield. The following day Steps Bridge was visited, the roundabout route taken by the coaches being much appreciated, especially by those who were paying their first visit to Devonshire. Tuesday's excursion was on foot to Stoke Woods, not far from Thomas Hall. Some parts of the wood were distinctly damp, but apparently the rain was too recent to have had the desired effect, for agarics were scarce and nearly all of small size. On Wednesday, what remained of the morning when the coaches made their first stop was spent in exploring Woodbury Fort, an old British earthwork, now planted with beech and conifers. Some fine specimens of *Gomphidius rutilans* (=*viscidus*), in all stages of development, were found, and a handful of other agarics were collected, of which the only one in any abundance was *Marasmius dryophilus*. After lunch the coaches proceeded to Branscombe, where a few members wisely decided that a walk to the beach was preferable to fungus hunting, for those who stayed had somewhat of a scramble and very few specimens to reward their efforts. The final foray was in the woods of Newton St Cyres, where collecting was comparatively successful. It was particularly interesting to find *Femsjonia luteo-alba*, recorded for this district at the Exeter Foray of 1901.

Although the larger fungi were far from abundant in all the areas explored, a fair number of species were collected during the week. Some fine young fruit bodies of *Fistulina hepatica*, brought in from one of the excursions, were served to the staff of Thomas Hall at lunch the following day, and pronounced good eating. At dinner crisply fried slices of *Polyporus sulphureus* appeared, but had a somewhat mixed reception. Several good specimens of *Cyathus striatus* were found, and examined with interest by a number of members who had not previously seen any of the bird's nest fungi. In several of the woods were numerous logs showing the characteristic green stain produced by *Chlorosplenium aeruginosum* and a fair number of them showed also typical apothecia.

A fine collection of microfungi was amassed during the week, and a continuous display of the more interesting finds, arranged by Mr S. J. Hughes, and consisting of specimens, slides, and rough drawings, was much appreciated.

The Foray Committee takes this opportunity of expressing its thanks to Dr A. S. Boughey, who made all the arrangements for the Foray and ensured their smooth running; to Professor J. Caldwell for the use of his departmental equipment and for conducting a tour of the University grounds; and to the Wardens of Duryard House and Thomas Hall for the excellent accommodation provided.

List of species recorded

B. = Branscombe; *Bi.* = Bickleigh; *E.* = Exeter; *N.* = Newton St Cyres; *S.* = Stoke Woods; *S.B.* = Steps Bridge; *Se.* = Sellers Wood; *W.* = Woodbury Fort; *U.* = University College Grounds, Garden of Thomas Hall, etc.

PHYCOMYCETES

Bremia lactucae Regel, on lettuce, *U.*

PYRENOMYCETES

Anthostoma turgidum (Pers. ex Fr.) Nits., on *Fagus*, *N.*
Anthostomella tomicoides Sacc., on *Rubus fruticosus*, *S.B.*
Apioporthe vepris (de Laer.) Weymeyer, on *Rubus fruticosus*, *S.B.*
Berlesiella nigerrima (Blox. ex Curr.) Sacc., on *Diatrype stigma*, on *Acer pseudoplatanus* wood *U.*, on *Diatrype stigma* on *Hedera helix* wood, *Se.*
Calyculosphaeria tristis (Fuck.) Fitzpatrick, on *Acer pseudoplatanus* assoc. with *Diatrype stigma*, *U.*
Chaetomium cochliodes Pall., on *Gunnera scabra* petioles, *U.*, elatum Kunze & Schmidt ex Fr., on wheat straw, *N.*
Chaetosphaeria callimorpha (Mont.) Sacc., on *Rubus fruticosus*, *S.B.*, *U.*, innumera (B. & Br.) Tul., on *Quercus* wood, *S.B.*, phaeostroma (Dur. & Mont.) Fuckel, on *Acer pseudoplatanus* wood assoc. with *Diatrype stigma*, *U.*, on *Acer campestre* branches assoc. with *Diatrype stigma*, *Se.*
Diaporthe decedens (Fr.) Fuck., on *Corylus avellana*, *S.B.*, eres Nits., on *Ulex*, *S.B.*, leiphemia (Fr.) Sacc., on *Quercus*, *S.*, *W.*, syngenesia (Fr.) Fuck., on *Rhamnus frangula*, *S.B.*
Diatrypella quercina (Pers. ex Fr.) Cke., on *Castanea sativa*, *S.*
Erysiphe cichoracearum DC., on *Plantago major* leaves, *N.*, polygoni DC., on *Heracleum sphondylium*, *U.*, on *Polygonum Sp.*, *N.*
Gibberella pulicaris (Fr.) Sacc., on *Pinus sylvestris* leaves, *W.*
Gloniopsis levantica Rehm, on *Ulex europaeus* wood, *S.B.*, *Quercus* wood, *Se.*, *Rubus fruticosus*, *N.*, *Fraxinus*, *N.*, *Se.*, *Prunus spinosa*, *N.*, *Corylus*, *S.B.*
Gnomonia rubi (Rehm) Wint., on *Rubus fruticosus*, *U.*
Hypoderma virgultorum DC. ex Sacc., on *Rubus fruticosus*, *S.B.*, *U.*, *W.*
Hypoxylon fuscum (Pers. ex Fr.) Fr., on *Corylus*, *Se.*, *Alnus glutinosa*, *S.B.*, howeianum Peck, on *Corylus avellana*, *Se.*, *S.B.*, multiforme (Fr.) Fr., on *Fraxinus*, *N.*, *Betula* bark, *S.B.*, wood, *N.*, rubiginosum (Pers. ex Fr.) Fr., on *Fraxinus excelsior* wood, *N.*, *S.*, rutilum Tul., on *Ilex*, *U.*, serpens (Pers. ex Fr.) Fr., on *Fraxinus* and *Corylus*, *S.B.*
Hysterium angustatum (A. & S. ex Fr.) Chev., on *Rhamnus frangula*, *S.B.*, pulicare Pers. ex Fr., on *Betula*, *S.B.*
Lasiosphaeria hirsuta (Fr.) Ces. & de Not., on *Fraxinus excelsior* wood, *U.*, *Trametes mollis*, 'Devon', and wood, *N.*, *U.*, spermoides (Hoffm. ex Fr.) de Not., on *Fraxinus excelsior* wood, *N.*, strigosa (A. & S. ex Fr.) Sacc., on wood, *N.*

Lophiotrema praemorsum (Lasch) Sacc. on *Ulex europaeus* bark, *S.B.*
Lophodermium cladophilum (Lev.) Rehm, on *Vaccinium*, *E.*, pinastri (Schrad. ex Fr.) Chev., on *Pinus* leaves, *W.*
Massaria inquinans (Tode ex Fr.) Ces. & de Not., on *Acer pseudoplatanus* wood, *S.*
Melanconis alni Tul., on *Alnus* twigs, *S.B.*, stilbostoma (Fr.) Tul., on *Betula*, *S.B.*, thelebola (Fr.) Sacc., on *Alnus* twigs, *S.B.*
Melanomma fuscidulum Sacc., on *Fraxinus* wood, *Se.*, *Rhamnus frangula* wood, *S.B.*, pulvis pyrius (Pers. ex Fr.) Fuckel, on *Ribes nigrum*, *U.*, *Ulmus*, *U.*, subdispersum (Karst.) Berl. & Vogl. on *Betula* bark, *S.B.*
Melanopsomella inequalis (Grove) v. Höhn., on wood, *Se.*
Nectria cinnabarina (Tode ex Fr.) Fr., on *Acer pseudoplatanus*, *N.*, coccinia (Pers. ex Fr.) Fr., on *Ilex aquifolium*, *Se.*, inventa Pethyb. (conidial), on *Solanum tuberosum*, *N.*, punicea (Kunze & Schum. ex Fr.) Fr., on *Fagus sylvatica* bark, *W.*, sinopica (Fr.) Fr., on *Hedera helix*, *S.B.*
Phyllactinia corylea (Pers.) Karst., on *Fraxinus*, *S.B.*
Podospora caudata (Curr.) A. L. Smith, on *Quercus* wood, *S.B.*
Quaternaria quaternata (Pers. ex Fr.) Schroet., on *Fagus*, *N.*
Rosellinia aquila (Fr.) de Not., on *Corylus avellana*, *N.*, *Acer pseudoplatanus*, *U.*, wood, *U.*, mammiformis (Pers. ex Fr.) Ces. & de Not., on *Hedera helix*, *Se.*
Systremma ulmi (Duval ex Fr.) Theiss. & Syd., on *Ulmus* leaves, *U.*
Trichosphaeria myriocarpa (Fr.) Petr. & Syd., on *Betula*, *S.B.*
Tympanopsis euomphala (Berk. & Curt.) Starb., on *Fraxinus excelsior* wood assoc. with *Hypoxylon rubiginosum*, *Se.*
Uncinula aceris (DC.) Sacc., on *Acer pseudoplatanus*, *S.*
Ustulina deusta (Hoffm. ex Fr.) Petr., on *Fagus sylvatica* (dead), *W.*
Valsa ambiens (Pers. ex Fr.) Fr., on *Rubus fruticosus*, *S.B.*
Xylaria longipes Nits, on *Acer pseudoplatanus* wood, *S.*, *U.*, polymorpha (Pers. ex Fr.) Grev., on wood, *S.*, *U.*
Zignoella pulviscula (Curr.) Sacc., on *Fagus*, *W.*

DISCOMYCETES

Arachnopeziza aurata Fuck., *S.*
Ascobolus purpurascens Pers., on cow dung, *B.*
Cenangium pulveraceum (A. & S.) Fr., on *Hedera helix* wood, *U.*, *Ulex europaeus*, *S.B.*
Chlorosplenium aeruginosum (Fr.) de Not., on *Fraxinus excelsior*, *N.*, *Quercus* and *Fraxinus*, *N.*, *S.*, *S.B.*
Ciliaria scutellata (Linn.) Quél., on rotten wood, *N.*
Colpoma quercina (Pers.), on *Quercus*, *S.B.*
Coryne sarcoides (Jacq.) Wallr. Tul., on *Fraxinus*, *N.*
Dasyscypha calycina (Schum.) Fuck., on *Abies*, *U.*, clandestina (Bull.) Fuck., on *Rubus*, *U.*, nivea (Hedw.) Sacc., *S.*, virginea (Batsch.) Fuck., on *Ulex* and other wood, *S.*, *W.*
Fabraea ranunculi (Fr.) Karst., on *Ranunculus acris* and *R. repens*, *N.*
Helotium cyathoideum (Bull.) Karst., on *Heracleum*, *U.*, fructigenum (Bull.) Fuck., *B.*, phyllophilum (Desm.) Karst., on *Fagus* leaves, *W.*, scutula (Pers.) Karst., *U.*, virgultorum Karst., *B.*
Hyaloscypha dematiicola (B. & Br.) Nannfeldt, on *Quercus* wood, *S.*, hyalina (Pers.) Boud., *S.*
Lachnum cerinum (Pers.) Nannf., on *Quercus* wood, *Se.*
Mollisia cinerea (Batsch.) Fr., abundant everywhere.
Orbilia xanthostigma Fr., abundant everywhere.
Peziza aurantia Pers., *Bi.*
Phacidium multivalve (DC.) Kunze & Schm., on *Ilex aquifolium* leaves, *S.B.*
Polydesmia pruinosa (B. & Br.) Boud., on *Hypoxylon multiforme* and on *Betula*, *S.B.*
Propolis versicolor Fr., on *Quercus* wood., *S.B.*
Pseudopeziza ranunculi (Wallr.) Fuck., on *Ranunculus repens*, *N.*
Rhytisma acerinum (Pers.) Fr. ex Fr., on *Acer pseudoplatanus* leaves, *S.*

BASIDIOMYCETES

Ustilaginales

Urocystis anemones (Pers.) Wint., on *Ranunculus repens*, *N.*

Uredinales

Coleosporium euphrasiae (Schum.) Wint., on *Odontites rubra*, *N.*, rhinanthacearum Lév., on *O. rubra*, *N.*

Kuehneola albida Magnus, on *Rubus fruticosus*, *S.*, uredinis (Link) Arth., on *Rubus fruticosus*, *S.*

Melampsora hypericorum (DC.) Wint., on *Hypericum androsaemum*, *N.*

Melampsoridium betulinum (Pers.) Kleb., on *Betula pendula*, *S.B.*

Phragmidium disciflorum James, on *Rosa canina*, *N.*, mucronatum (Pers.) Schlecht., on *Rosa*, *N.*, rubi (Pers.) Wint., on *Rubus fruticosus*, *S.*, *U.*, violaceum Wint., on *Rubus fruticosus*, *S.*

Puccinia coronata Corda, on *Agropyron* sp., *U.*, glumarum Erikss. & Henn., on *Brachypodium sylvaticum*, *S.*, iridis (DC.) Wallr., on *Iris*, *B.*, lapsanae Fuckel, on *Lapsana communis*, *N.*, menthae Pers., on *Mentha arvensis*, *N.*, primulae Duby, on *Primula acaulis*, *Se.*, taraxaci Plowr., on *Taraxacum officinale*, *N.*

Uromyces genistae-tinctoriae (Pers.) Wint., on *Sarothamnus scoparius*, *S.B.*, *Cytisus*, *Se.*, rumicis Wint., on *Rumex* sp., *N.*

Agaricales

Amanita phalloides (Vaill.) Fr., *S.B.*

Amanitopsis fulva (Schaeff.) W. G. Sm., *S.B.*, *W.*

Anellaria semi-ovata (Sow. ex Fr.) Pearson & Dennis, *W.*

Armillaria mellea (Vahl) Fr., *N.*

Bolbitius fragilis (Linn.) Fr., *N.*

Boletus chrysenteron (Bull.) Fr., *N.*, edulis (Bull.) Fr., *S.B.*, erythropus Fr., *N.*, scaber (Bull.) Fr., *S.B.*, variegatus (Swartz) Fr., *S.*, versicolor Rostk., *N.*

Clitocybe infundibuliformis (Schaeff.) Fr., *N.*, *S.*

Clitopilus prunulus (Scop.) Fr., *N.*

Collybia fusipes (Bull.) Berk., *N.*, *S.*, *S.B.*, radicata (Rehl.) Berk., *Bi.*, *N.*

Coprinus cinereus (Schaeff.) Fr., *S.*, lagopus Fr., *N.*, niveus (Pers.) Fr., *U.*, plicatilis (Curt.) Fr., *N.*, *S.*, *U.*, *W.*

Crepidotus variabilis (Pers.) Fr., *N.*, *W.*

Galera tenera (Schaeff.) Fr., *B.*, *N.*

Gomphidius rutilis (Schaeff.) Fr., *W.*

Hypholoma candolleanum Fr., *N.*, velutinum (Pers.) Fr., *N.*, *U.*

Laccaria tortilis (Bolt.) Cke., *N.*

Lactarius cyathula Fr., *S.B.*, deliciosus (Linn.) Fr., *W.*, vellereus Fr., *N.*, *W.*

Marasmius androsaceus (Linn.) Fr., *B.*, *S.*, *W.*, confluens (Pers.) Karst., *U.*, *W.*, dryophilus (Bull.) Karst., *N.*, *S.B.*, *W.*, peronatus (Bolt.) Fr., *Bi.*, *U.*, ramealis (Bull.) Fr., *B.*, *N.*, *U.*, rotula (Scop.) Fr., *S.*, *U.*, vaillantii (Pers.) Fr., *B.*

Mycena haematopus (Pers.) Fr., *S.B.*, hiemalis (Osbeck) Fr., *S.*, rorida Fr., *S.B.*, rubromarginata, Fr., *S.B.*, *W.*, sanguinolenta (A. & S.) Fr., *N.*, *S.B.*, *U.*, *W.*, stylobates (Pers.) Fr., *N.*, *S.B.*, *U.*

Naucoria escharoides Fr., *S.B.*, scolecina Fr., *S.B.*

Omphalia fibula (Bull.) Fr., *N.*, gracilis Quél., *S.B.*

Panaeolus campanulatus (Linn.) Fr. var. sphinctrinum (Fr.) Bres., *U.*

Panus stipticus (Bull.) Fr., *W.*, torulosus (Pers.) Fr., *U.*

Paxillus involutus (Batsch.) Fr., *S.B.*

Pholiota togularis (Bull.) Fr., *U.*

Pleurotus cornucopiae (Paulet) Pers., *U.*, palmatus (Bull.) Fr., *B.*

Pluteolus aleuriatus Fr., *N.*

Pluteus cervinus (Schaeff.) Fr., *N.*, *S.*, *S.B.*, *W.*, nanus (Pers.) Fr., *S.*, *U.*, salicinus (Pers.) Fr., *U.*

Psaliota campestris (Linn.) Fr., *U.*, *W.*, xanthoderma Genev. var. obscurata Maire, *U.*

Psathyrella fibrillosa (Pers. ex Fr.) Pearson & Dennis, *S.B.*

Psilocybe cernua (Vahl) Fr., *U.*, ericacea (Pers.) Fr., *W.*
Russula cyanoxantha (Schaeff.) Fr., *S.B.*, *W.*, emetica (Schaeff.) Fr., *W.*, nigricans (Bull.) Fr., *W.*, vesca Fr., *W.*
Stropharia semiglobata (Batsch.) Fr., *B.*, *N.*, *S.*
Tubaria furfuracea (Pers.) Gillet, *S.B.*
Volvaria bombycina (Schaeff.) Fr., *Bi.*

Aphyllophorales

Acia fusco-atra (Fr.) Pat., *S.B.*, uda (Fr.) Bourd. & Galz., *N.*, *S.B.*, *U.*
Clavaria cinerea (Bull.) Fr., *W.*, cristata (Holmsk.) Fr., *S.B.*
Coniophora puteana (Schum.) Karst., *U.*
Corticium confine B. & G., *S.B.*, praetermissum Karst., *N.*, *S.*, *S.B.*, *W.*, sambuci (Pers.) Fr., *S.*, on *Acer pseudoplatanus* wood, *U.*, sulphureum (Pers. ex Fr.) Fr., *W.*
Daedalea quercina (Linn.) Fr., *N.*
Fistulina hepatica (Huds.) Fr., *N.*, *S.*, *S.B.*
Fomes annosus (Fr.) Cke., *U.*, *W.*, ferruginosus (Schrad. ex Fr.) Mass., *N.*, fraxineus (Fr.) Cke., *B.*, ribis (Schum. ex Fr.) Gill., *B.*, on Elder, *S.B.*, ulmarius (Sow. ex Fr.) Sacc., *U.*
Ganoderma lucidum (Leyss.) Karst., on Holm Oak, *N.*
Grandinia brinkmanni (Bres.) B. & G., *S.*, farinacea (Pers.) B. & G., *N.*, granulosa (Pers. ex Fr.) Fr., *S.B.*
Hydnum auriscalpium (Linn. ex Fr.) Fr., *W.*
Hymenochaete corrugata (Fr.) Lév., *N.*, *S.B.*, *U.*, rubiginosa (Fr.) Lév., *S.*
Irpex obliquus (Schrad.) Fr., *S.*
Merulius rufus (Pers.) Fr., *N.*, *S.*, tremellosus (Schrad.) Fr., *S.*
Pellicularia pruinata (Bres.) Rogers (conidial), on *Ulex europaeus* wood, *S.B.*, vaga (B. & C.) Rogers, *N.*
Peniophora byssoidea (Pers.) v. H. & L., forma tomentella Bres., *W.*, candida Lyman, *U.* (associated with *Aegerita candida*), *S.B.* (*Aegerita* stage only), cinerea (Fr.) Cke., *S.B.*, on *Hedera helix* wood, *U.*, *Corylus avellana*, *U.*, cremea Bres., *S.*, *S.B.*, hydnoides (Cooke) Mass., *S.B.*, *W.*, roumeguerii (Bres.) v. H. & L., *N.*, *S.*, setigera (Fr.) v. H. & L., *S.B.*
Phlebia merismoides Fr., *S.B.*, *U.*
Pistillaria micans (Pers.) Fr., *U.*
Polyporus betulinus (Bull.) Fr., *N.*, *S.B.*, caesius (Schrad.) Fr., *N.*, *S.*, *U.*, *W.*, hispidus (Bull.) Fr., *B.*, schweinitzii Fr., *U.*, *W.*, squamosus (Huds.) Fr. forma forguignoni (Quél.) Pilat, *N.*, *S.B.*, sulphureus (Bull.) Fr., *S.*, *W.*
Polystictus abietinus (Dicks.) Fr., *W.*, versicolor (Linn.) Fr., *N.*, *S.*, *U.*
Poria mucida (Pers.) Fr., *N.*, sanguinolenta (A. & S.) Fr., *W.*
Solenia anomala (Pers.) Fr., *U.*, candida (Pers.) Fr., *B.*
Sparassis crispa (Wulf.) Fr., *S.*
Stereum gausapatum Fr., N., hirsutum (Willd.) Fr., *B.*, *N.*, *S.*, *U.*, purpureum (Pers.) Fr., *U.*, rugosum (Pers.) Fr., *N.*, on *Euonymus* sp. *U.*, sanguinolentum Fr., *W.*
Thelephora laciniata (Pers.) Fr., *W.*
Trametes mollis (Sommerf.) Fr., *N.*, *U.*, rubescens (A. & S.) Fr., *S.B.*, *W.*

Auriculariales

Auricularia auricula-judae (Linn.) Schroet., *N.*, *S.B.*, *U.*, mesenterica (Dicks.) Fr., *U.*, *W.*

Tremellales

Dacryomyces deliquescens (Bull.) Duby, *S.*, *U.*
Exidia glandulosa (Bull.) Fr., *W.*, nucleata (Schwein.) Rea, *B.*, *W.*
Femsjonia luteo-alba Fr., *N.*
Tremella mesenterica (Retz.) Fr., *S.B.*

Calocerales

Calocera viscosa (Pers.) Fr., *S.*, *W.*

GASTEROMYCETALES

Cyathus striatus (Huds.) Pers., *S.*, *U.*, *W.*
Geaster bryantii Berk., *S.*, lageniformis Vitt., *U.*
Phallus impudicus (Linn.) Pers., *W.*
Scleroderma aurantium Pers., *S.*, *S.B.*, verrucosum (Vaill.) Pers., *N.*, *S.B.*

HYPHOMYCETES

Acrothecium delicatulum B. & Br., on wood, *U.*, and *Fraxinus excelsior* wood, *Se.*
Alternaria tenuis Nees ex Wallr. (group), on *Mercurialis perennis*, *Se.*
Bactrodesmium caulincola (Corda) Grove var. pellucidum Grove, on wood, *U.*
Botryosporium pulchrum Corda, on *Urtica dioica* stems, *U.*
Brachysporium apicale (B. & Br.) Sacc., on *Quercus* wood, *S.*, *S.B.*, bloxami (Cooke) Sacc., on *Fagus sylvatica* wood, *W.*, *Quercus* wood, *S.B.*, obovatum (Berk.) Sacc., on *Acer pseudoplatanus* wood, *U.*
Cercospora depazeoides (Desm.) Sacc., on *Sambucus nigra* leaves, *S.*, mercurialis Pass. on *Mercurialis perennis*, *Se.*
Cladosporium herbarum Link ex Fr., on *M. perennis*, *Se.*
Clasterosporium abruptum (B. & Br.) Sacc., on *Fraxinus excelsior* wood, *Se.*, *U.*, on *Acer pseudoplatanus* wood, *U.*, fasciculare (Corda) Sacc., on wood, *N.*, *Quercus* wood, *S.B.*
Cristulariella depraedans (Cooke) v. Höhn., on *Acer pseudoplatanus*, *S.*
Cryptocoryneum hysterioides (Corda) Peyronel, on *Corylus avellana* wood, *Se.*,*S.B.*, *Fraxinus excelsior* bark, *Se.*, *Quercus* wood, *S.B.*
Cylindrodendrum album Bon., on *Ilex aquifolium* leaves and other plant debris, *W.*
Cytospora laurocerasi Fuck., *U.*
Dendryphiella interseminata (Berk. & Rav.) Bub. & Ran., on *Heracleum sphondylium* dead stems, *N.*
Dendryphion comosum Wallr., on *Heracleum sphondylium* dead stems, *U.*, curtum Berk. & Br., on *Heracleum*, *U.*
Epicoccum neglectum Desm., on *Mercurialis*, *B.*, purpurascens Ehrenb. ex Wallr., on *Alnus glutinosa* leaves, *N.*, *Mercurialis perennis*, *Se.*
Fusicladium depressum (B. & Br.) Sacc., on *Angelica sylvestris*, *U.*
Fusidium viride Grove, on *Heracleum sphondylium* and *Gunnera scabra*, *U.*
Gliocladium luteolum v. Höhn., on wood, *S.*
Haplaria grisea Link ex Chev., on *Quercus* wood, *S.*, *S.B.*
Haplographium bicolor Grove, on *Quercus* wood, *S.*, *Rubus*, *U.*
Helicosporium vegetum Nees, on *Betula* wood, *S.B.*
Helminthosporium apiculatum Corda, on *Corylus avellana* wood, *S.*, *Se.*, *Rubus fruticosus*, *N.*, longipilum Corda, on *Betula*, *S.B.*, simplex Kunze ex Fr., on *Hedera helix* wood, *U.*, *Fagus sylvatica* wood, *Se.*, *Ilex aquifolium* wood, *W.*, *Acer pseudoplatanus*, *S.*, wheat stubble, *N.*, smithii Berk. & Br., on *Ilex aquifolium*, *Se.*, *U.*, velutinum Link ex Fr. on *Euonymous* sp. twigs, *S.*, *Ribes nigrum* branches, *U.*, *Ilex aquifolium*, *S.*
Isaria umbrina Pers., ex Wallr., on *Corylus avellana* bark, *S.B.*
Menispora caesia Preuss on *Hedera helix* wood, *U.*, *Quercus* wood, *S.B.*, ciliata Corda on *Quercus* bark, *S.B.*
Oidium aureum Link ex Fr., on *Fraxinus excelsior*, *Se.*, rotten wood, *S.*, *Se.*, conspersum (Link) Linder, on rotten wood and bark, *S.*
Passalora depressa (B. & Br.) v. Höhn., on *Angelica sylvestris*, *U.*
Periconia byssoides Pers. ex Corda, on *Mercurialis perennis*, *B.*, *Se.*
Phaeoisaria cornui (Bain.) Mason, on *Diatrype stigma* on *Acer pseudoplatanus*, *U.*
Ramularia primulae Thüm., on *Primula vulgaris*, *Se.*, schulzeri Bauml., on *Lotus corniculatus*, *N.*, variabilis Fuck., on *Digitalis purpurea*, *N.*
Septocylindrium pallidum Grove, on *Diatrype stigma* on: *Acer pseudoplatanus* wood, *U.*, *Fraxinus excelsior*, *U.*, *Ilex aquifolium*, *U.*, *Se.*, *Hedera helix*, *U.*
Speira toruloides Corda, on wood, *N.*
Spicaria elegans (Corda) Harz, on *Quercus* wood, *S.B.*, bark of log, *S.*
Sporidesmium paradoxum Corda, on *Betula* bark, *S.B.*
Sporochisma insigne Sacc., Rouss. & Bomm., on *Corylus avellana* bark, *S.B.*, mirabile Berk. & Br., on wood, *N.*

Stachybotrys atra Corda, on wheat straw, *N.*, dichroa Grove, on dead herbaceous stems, *U.*
Tetraploa aristata Berk. & Br. on wheat stubble, *N.*,
Triposporium elegans Corda, on *Corylus avellana* wood, *N.*, wood, *S.B.*, on *Rubus fruticosus*, *S.B.*, *U.*
Torula herbarum Link ex Fr., on *Heracleum sphondylium*, *U.*
Verticicladium trifidum Preuss, on *Pinus sylvestris* leaves, *W.*
Verticillium apicale Berk. & Br., on *Quercus* wood, *S.B.*, candelabrum Bon., on *Quercus S.B.*

COELOMYCETES

Diplodia pinea (Desm.) Kickx, on *Pinus sylvestris* leaves, *W.*
Phyllosticta campestria Pass., on *Acer campestre* leaves, *Se.*
Septoria cornicola Desm., on *Cornus sanguinea* leaves, *Se.*
Stagonospora typhoidearum (Desm.) Sacc., on *Typha* leaves, *U.*
Vermicularia volutella Grove, on *Rubus fruticosus*, *S.B.*

Specimens of most of the Pyrenomycetes and Hyphomycetes have been deposited in the herbarium of the Commonwealth Mycological Institute, Kew.

LONDON FORAYS

Five forays were held in the London area: Mad Bess Wood, Ruislip, 27 September; Oxshott, 28 September; Ashtead, 4 October; Bookham Common, 5 October; and Epping Forest, 11 October. The prolonged drought had affected all the areas visited and, whilst the older members found a certain fascination in hunting for the odd specimens of even the commonest fungi, the many students who were attending their first foray were obviously disappointed with the meagre collections. As was to be expected, lignicolous species accounted for a large proportion of the finds. After the Ashtead Foray members proceeded to Epsom College, where, by kind permission of the Headmaster, tea was provided and the biology laboratory thrown open for examination and display of specimens.

SPRING FORAY

The 1947 Spring Foray was held in the Reading area, with headquarters in the Botanical Department of the University (by kind permission of Professor T. Harris), from Saturday, 10 May to Monday, 12 May.

PROCEEDINGS, 1948

MEETINGS

Meeting held in the Department of Bacteriology, London School of Hygiene and Tropical Medicine, at 2.15 p.m., 9 April 1948. Dr J. Ramsbottom, Vice-President, in the Chair.

The meeting took the form of a demonstration of certain aspects of Medical Mycology, with slides, specimens and photographs. It was introduced by Dr J. T. Duncan, who commented on dimorphism amongst fungi pathogenic to human beings. Mrs J. Walker showed cultures and microscope preparations of Dermatophytes, and Dr Clara Warren exhibited photographs of dermatomycoses and demonstrated the test of fluorescence to ultra-violet light used in the diagnosis of certain forms of ringworm infection of hairs.

Meeting held at the Botany School, Cambridge, 11.0 a.m., 17 July 1948. The President, Prof. A. E. Muskett, in the Chair.

J. Rishbeth. *Fomes annosus* on pines in East Anglia.
E. G. Bollard. The parasitism of the genus *Mastigosporium*.
J. G. Manners. Physiologic specialization of *Puccinia glumarum* in Great Britain.
A. R. Loveless. Some aspects of the biology of *Sclerotinia trifoliorum*.
D. Rudd Jones. Bacterial rots of stored potatoes.
C. E. Harrold. On the re-discovery of *Eremascus albus*.
J. H. Warcup. Some observations on the fungi of Breckland soils.

Meeting held in the Department of Botany, Imperial College of Science and Technology, South Kensington, at 11.0 a.m., 22 October 1948. The President, Prof. A. E. Muskett, in the Chair.

Miss M. J. Smieton, R. K. S. Wood, F. J. Newhook. The *Rhizoctonia* disease of lettuce. (Introduced by Prof. W. Brown.)
J. R. Booer. White Rot disease of onions.
W. G. Keyworth. Recent research in *Verticillium* wilts.
H. F. Dovaston. Influence of soil conditions on *Fusarium* Foot Rot of oats.

Meeting held in the Department of Biology, Chelsea Polytechnic, London, S.W. 3, at 2.15 p.m., 4 December 1948. The President, Prof. A. E. Muskett, in the Chair.

G. C. M. Harris and Miss L. Bannan. The control of variation in *Penicillium chrysogenum*, Q. 176.
C. T. Ingold. On the genus *Resticularia*.
S. A. Hutchinson. The preservation of Fungi in plastics.

ANNUAL GENERAL MEETING

The fifty-second Annual General Meeting was held in the Department of Biology, Chelsea Polytechnic, London, S.W. 3, at 12 noon on Saturday, 4 December 1948, with the President, Prof. A. E. Muskett, in the Chair.

After the Minutes of the previous Annual General Meeting had been read and signed the President recorded with deep regret the deaths during the year of three members, the Rev. M. C. Potter, one of the original members of the Society, G. H. Pethybridge, and A. Smith. He then briefly reviewed the year's activities.

The Treasurer, W. Buddin, submitted his annual statement and again emphasized the high cost of proof corrections.

The following Officers and new members of the Council for 1949 were then elected: *President*, W. P. K. Findlay; *Vice-Presidents*, N. C. Preston, and the two past-Presidents, A. E. Muskett and C. G. C. Chesters; *Secretary*, C. J. Hickman; *Foray Secretary*, G. C. Ainsworth; *Treasurer*, W. Buddin; *Editors*, B. Barnes and W. C. Moore; *Members of the Council*, P. H. Gregory, Miss G. Waterhouse, and R. K. S. Wood (to replace S. J. Hughes, H. E. Croxall and F. A. Sowter). J. Colhoun, W. G. Keyworth, Miss L. E. Hawker and S. D. Garrett were elected as new members of the Plant Pathology Committee.

On a motion from the Chair, Prof. F. T. Brooks was elected as an Honorary Member. Twelve new Members and one Associate were elected, making a total of sixty-three new Members and ten new Associates for the year. Two Associates became Members during the year, bringing the total membership to 462 (12 Honorary Members) and the number of Associates to 49.

Proposed by J. Ramsbottom, a vote of thanks was passed to Prof. Muskett for his work on behalf of the Society during his year of office.

After the programme for 1949 had been discussed the following alteration in the Rules was adopted: Rule 7, for 'Secretaries' read 'Secretary'.

On a motion from the Chair it was decided that a resolution supporting the principle of *nomina specifica rejicienda* should be forwarded in the name of the Society to the appropriate Committee of the 1950 International Botanical Congress.

The proposal for the establishment of an International Herbarium of Plant Pathogenic Fungi (outlined in vol. **31** of the *Transactions*, pp. 346–348) was then discussed and received general support.

In conclusion, the Secretary drew attention to a notice inviting subscriptions to a volume of mycological papers being produced in commemoration of Dr René Maire's seventieth birthday, and to a notice of the Vth International Congress of Comparative Pathology, to be held in Istanbul in May, 1949.

C. J. HICKMAN, *Secretary*

RECEIPTS AND PAYMENTS

for the year ended 30 June 1948

		£ s. d.	£ s. d.
1947 1 July	Balance brought forward		528 15 10
12 Aug.	Direct sale of *Transactions*		2 0 0
29 Dec.	Grant from Royal Society towards production of Jubilee Volume ...		150 0 0
1948 30 June	Sales:		
	Diseases of British Grasses		8 17 8
	Phytopathological Classic		12 0 6
	Donations:		
	Printing Fund	7 18 0	
	Hospitality Fund	27 4 0	
			35 2 0
	Interest:		
	Capital Reserve Fund	36 0 0	
	Post Office Account	3 2 6	
			39 2 6
	Subscriptions:		
	Arrears	22 0 0	
	Current	422 4 0	
	In advance	14 5 0	
	Associates	7 15 0	
			466 4 0
			£1242 2 6

		£ s. d.	£ s. d.
1947 15 Sept.	Cheesman Fund Grant		5 5 0
1948 24 June	Purchase of 50 copies of *Phytopathological* Classic No. 8 (Berkeley) ...		18 7 6
30 June	Subscriptions:		
	Refunded	2 0 0	
	Transferred to C.U.P.	1 4 6	
	Woolhope Club	1 0 0	
	Biological Council	1 1 0	
			5 5 6
	Postages, cheque, stamps, stationery, etc.		
	Secretary	16 12 11	
	Foray Secretary	1 10 3	
	Secretary Plant Path. Com. ...	3 17 9	
	Editors	4 1 1	
	Treasurer	5 14 2	
			31 16 2
	Sundry Printing		23 7 5
	Fees for Meetings		4 5 0
	Sundry Secretarial Expenses ...		4 0 0
	Balance:		
	At Midland Bank ... 466 10 3		
	Less unpresented cheques 17 13 0		
		448 17 3	
	Held in Post Office Account ...	703 2 6	
	Petty Cash in Treasurer's hands	5 2	
		1152 4 11	
	Less overdraft at New York Bank	2 9 0	
			*1149 15 11
			£1242 2 6

* From this balance must be met most of the cost of production of the Jubilee Volume (xxx) and Vol. xxxi of *Transactions*.

CAPITAL RESERVE FUND ACCOUNT (incorporating the Cheesman and Printing Funds)

Nominal values of Stock { 3½% War Stock ... £500; 3% Defence Bonds ... £200; 2½% Defence Bonds ... £500 } Total £1200

Examined and found correct, W. C. MOORE, 30 July 1948

WALTER BUDDIN, *Hon. Treasurer*

THE FLATFORD MILL FORAY

7–9 May 1948

By G. C. AINSWORTH, *Foray Secretary*

The twenty-ninth Spring Foray was held at the Flatford Mill Field Centre of the Council for the Promotion of Field Studies from Friday, 7 May to Sunday, 9 May 1948.

Flatford Mill is situated in the parish of East Bergholt on the Suffolk bank of the River Stour about four miles from Manningtree station. Downstream the river is tidal and the valley quickly opens out into extensive salt marshes. Upstream towards Dedham (two miles away) there are fresh-water marshes and on the higher ground areas of woodland. The Centre caters for students of diverse interests, and the old mill and adjacent buildings now comprise a well-equipped laboratory, lecture rooms, library and common room, together with a refectory and dormitory accommodation. The Society is greatly indebted to the Warden, Dr E. A. R. Ennion, for his warm welcome and for all that he and his staff did as guides and advisors to make the foray both pleasurable and mycologically profitable.

Friday's excursion was to Dedham where a halt was made for lunch and liquid refreshment. The outward route was on the Suffolk side of the river, the return route on the Essex bank and among other interesting finds were *Clasterosporium caricinum*, which had previously only been reported in this country from Norfolk, and *Leptosphaeria sowerbyi* on dead *Scirpus lacustris* culms.

The next day the party went farther afield to the saltings and patches of woodland on the Essex bank of the Stour below Mistley when fine weather again added much to the day's enjoyment. *Puccinia adoxae* and *Uromyces scillae* were found in abundance, as was *Entyloma ficariae*. *Lasiosphaeria spermoides* was found in association with *Armillaria mellea* and a fine growth of *Monilia aurea* was noted.

On Sunday, 9 May, the higher ground to the north-west of the Centre was worked and though the woods were dry the collections were up to the average for the spring.

A complete list of the species recorded is appended. Specimens of many have been deposited at the Centre, while others may be found in the herbarium of the Commonwealth Mycological Institute, Kew. Acknowledgement is made to all those who contributed to the list, and particularly to Mr and Mrs M. B. Ellis for many records and specimens, and to Dr R. W. G. Dennis who kindly examined collections of discomycetes.

List of the species recorded during the Foray

D = banks of Stour between Flatford and Dedham; *F* = woods and marshes near Flatford; *M* = Mistley.

PHYCOMYCETES

Entomophthorales

Entomophthora aphidis Hoffm., on an aphid on nettle, *D*.

ASCOMYCETES

Taphrinales

Protomyces microsporus Ung., on *Sium erectum*, *D.*

Discomycetes

Aleuria repanda (Fr.) Gill., *M.*
Ascobolus furfuraceus Fr., *F.*
Calloria fusarioides (Berk.) Fr., on *Urtica*, *F.*
Cheilymenia stercorea (Pers.) Boud., *F.*
Ciliaria scutellata (L.) Boud., *F.*
Dasyscypha virginea (Batsch) Fuckel, on *Quercus cerris*, *F.*
Lasiobolus equinus (Mill.) Karst.
Orbilia xanthostigma Fr., *F.*
Rutstroemia nervisequia (Schroet.) White, on *Alnus* (leaves), *D.*
Sclerotinia candolleana (Lév.) Fuckel, *D.*
Trichobelonium asteroma (Fuckel) Rehm, on *Carex riparia*, *D.*

Pyrenomycetes

Chaetomium elatum Fr., on wheat stubble, *D.*
Chaetosphaeria phaeostroma (Dur. & Mont.) Fuckel (with Diatrype stigma), on *Ilex*, *D.*, *M.*
Daldinia concentrica (Fr.) Ces. & de Not., on ash, *D.*, *M.*, elm, F.
Diaporthe leiphaemia (Fr.) Sacc., on *Quercus robur*, *F.*
Diatrype disciformis (Fr.) Fr., *F.*, stigma (Fr.) Fr., on *Corylus*, *F.*, *Fraxinus*, *M.*
Diatrypella quercina (Fr.) Cooke, F.
Didymosphaeria palustris (B. & Br.) Sacc., on *Carex acutiformis*, *F.*
Epichloe typhina (Fr.) Tul., on *Dactylis*, *D.*
Hypoxylon fuscum (Fr.) Fr., on *Alnus*, *D.*, *Corylus*, *M.*, *F.*, howeianum Peck, on *Corylus*, *F.*, multiforme (Fr.) Fr., on *Betula*, *F.*, rubiginosum (Fr.) Fr., on *Fraxinus*, *M.*, semi-immersum Nits., on *Sambucus*, *M.*, *Quercus*, *F.*, serpens (Fr.) Fr., *M.*
Lasiosphaeria hirsuta (Fr.) Ces. & de Not., *M.*, spermoides (Fr.) Ces. & de Not. (associated with Armillaria mellea rhizomorphs), *M.*
Leptosphaeria acuta (Fr.) Karst., on nettle, *F.*, arundinacea (Fr.) Sacc., on *Phragmites communis*, *F.*, (Leptosphaeria) culmicola (Fr.) Karst., on *Phalaris arundinacea* and wheat stubble, *D.*, nigrans (Desm.) Ces. & de Not., on wheat stubble, *D.*, sowerbyi (Fuckel) Sacc., on *Scirpus lacustris*, *D.*
Libertella blepharis A. L. Sm., on *Sorbus aucuparia*, *M.*
Melanopsamella inaequalis (Grove) v. Höhn., on *Fraxinus*, *M.*
Nectria punicea (Fr.) Fr., on ivy, F.
Ophiobolus eucryptus (B. & Br.) Sacc., on *Carex acutiformis*, F.
Ophiosphaeria gracilis (Niessl) Kirsch., on *Carex* and *Glyceria*, F.
Rosellinia aquila (Fr.) de Not., on *Ulmus* and *Ilex*, *M.*
Trichosphaeria myriocarpa (Fr.) Petrak & Syd., on *Fraxinus*, *M.*
Xylaria hypoxylon (Fr.) Grev., *M.*, *F.*

Hysteriales

Hysterium angustatum (Fr.) Grev., on *Fraxinus*, *M.*
Lophodermium arundinaceum (Fr.) Chev., on wheat stubble, *D.*
Phacidium multivalve (DC.) Kunze & Schm., *M.*, *F.*
Stegia ilicis Fr., *D.*, *M.*, *F.*

BASIDIOMYCETES

Uredinales

Melampsora rostrupii Wagner, on *Mercurialis perennis*, *F.*
Phragmidium fragariastri (DC.) Schroet., on *Potentilla sterilis*, *D.*, mucronatum Fr., D.
Puccinia adoxae Hedw. f., *M.*, caricis Reb., on *Carex* and *Urtica*, *F.*, epilobii DC., *D.*, lapsanae Fuckel, *D.*, malvacearum Mont., *D.*, persistens Plowr., on *Thalictrum flavum*, *D.*, scirpi DC., D., suaveolens Rost., *D.*, *M.*, taraxaci Plowr., *D.*, *F.*

Triphragmium ulmariae Wint., *F.*
Uromyces acetosae Schroet., *D.*, ficariae (Schum.) Lév., *D.*, *M.*, *F.*, poae Rabenh., on *Ranunculus* and *Poa*, *M.*, *D.*, scillarum Wint., *M.*

Ustilaginales

Entyloma fergussoni (B. & Br.) Plowr., on *Myosotis palustris*, *D.*, ficariae Berk., *M.*, *F.*
Ustilago longissima (Schlecht.) Meyer, *D.*, *F.*, violacea (Pers.) Rous., *F.*

Hymenomycetes

Amanitopsis vaginata (Bull.) Roze.
Armillaria mellea (Vahl) Fr., *M.*
Coprinus lagopus Fr., *F.*, micaceus (Bull.) Fr., *D.*, *M.*, radians (Desm.) Fr. (mycelium), *D.*
Hypholoma fasciculare (Huds.) Fr., *D.*
Marasmius oreades (Bolt.) Fr., *D.*, *M.*
Pluteus cervinus (Schaeff.) Fr., *M.*, *F.*
Psalliota campestris (L.) Fr., *M.*
Schizophyllum commune Fr., *F.*
Tricholoma gambosum Fr., *D.*
Ganoderma applanatum (Pers.) Pat., *F.*
Polyporus brumalis (Pers.) Fr., *F.*, caesius (Schrad.) Fr., *F.* squamosus (Huds.) Fr., *F.*
Polystictus versicolor (L.) Fr., *F.*
Auricularia auricula-judae (L.) Schroet., *M.*, *F.*
Irpex obliquus Fr., *M.*, *F.*
Stereum purpureum (Fr.) Fr., on blackthorn, *D.*, lime, *F.*

Gasteromycetes

Bovista nigrescens Pers., *D.*
Lycoperdon caelatum (Bull.) Fr., *D.*

FUNGI IMPERFECTI

Acrothecium simplex B. & Br., on *Urtica*, *F.*
Arthrinium puccinioides Fr., on *Carex*, *F.*
Botrytis cinerea Fr., *D.*
Clasterosporium caricinum Schw., on *Carex riparia*, *D.*
Colletotrichum graminicola (Ces.) Wils., on *Glyceria*, *D.*
Cytospora rhodophila Sacc., on *Rosa canina*, *M.*, rosarum Grev., *M.*
Dendryphium comosum Wallr., on *Urtica*, *F.*, laxum B. & Br., on wheat stubble, *D.*
Didymaria didyma (Unger) Schroet., on *Ranunculus repens*, *D.*
Epicoccum purpurascens Wallr., on *Carex* and *Typha*, *D.*
Helminthosporium simplex Fr., *F.*, *M.*, velutinum Fr., on *Hedera helix*, *F.*
Leptostroma spiraeinum Vesterg., on *Filipendula*, *F.*
Monilia aurea Auct., *M. F.*
Neottiospora caricinum Desm., *F.*
Ovularia obliqua (Cooke) Oudem., on *Rumex*, *D.*, *F.*
Pachnocybe grisea Berk., on *Quercus*, *F.*
Papularia sphaerosperma (Fr.) v. Höhn., on *Phragmites*, *F.*
Periconia byssoides Corda, on *Urtica*, *F.*
Phaeoisaria cornui (Bain.) Mason, *M.*
Phoma sambuciphila Oudem., *M.*
Phyllosticta hedericola Dur. & Mont., *D.*
Septoria caricola Sacc., *D.*, rubi Westend., *D.*
Stagonospora caricis (Oud.) Sacc., *D.*, sparganii All., *D.*, *F.*
Torula herbarum Fr., on wheat stubble, *D.*, *Urtica*, *F.*
Trichoderma viride Fr., *F.*

MYXOMYCETES

Arcyria denudata Wetts., *F.*
Ceratomyxa fructiculosa Macbr., *F.*
Lycogala epidendrum Adans., *M.*, *F.*
Reticularia lycoperdon Bull., *F.*

THE BELFAST FORAY

11–17 September 1948

By G. C. AINSWORTH, *Foray Secretary*

The forty-fourth Autumn Foray was held in Northern Ireland from Saturday, 11 September to Friday, 17 September 1948 with headquarters, by kind invitation of the President, Prof. A. E. Muskett, at the Department of Mycology and Plant Pathology of Queen's University, Belfast.

Most of the members from Great Britain crossed by the night boats and arrived in Belfast early on the Saturday morning when they were met by specially chartered buses which took them to hotels or to Riddel Hall, one of the University hostels, where most of the party of more than seventy members was accommodated.

Following lunch at Overseas House a visit was made to Belvoir Park on the outskirts of the city where, after a skirmish with press photographers, the area was worked and useful miscellaneous collections made.

There was heavy rain on Sunday morning but fortunately the weather cleared to allow a most enjoyable afternoon trip to the Glens of Antrim. Glenariff was traversed and among the more interesting finds was *Tuber puberulum*, a new record for Ireland, which was turned up by Dr P. H. Gregory's rake, a tool in much demand on succeeding excursions although no other species of truffle were uncovered. Tea was taken at the foot of the Glen and a memorable forty-mile drive home along the coast road followed.

On Monday, 13 September there was an excursion to Tollymore Park, Co. Down, where two parties took different routes across the wooded estate to the gardens of Tollymore House. The adjacent forestry nursery and the gardens yielded records of several pathogenic species. In the evening there was a business meeting at Headquarters, when an invitation from Dr W. H. Wilkins to hold the 1949 Autumn Foray at Oxford was welcomed. After Madame Marcelle Le Gal, who attended the Foray as a guest of the Society, had presented the Society with an inscribed copy of her important monograph on spore ornamentation in the Discomycetes, and Dr Gregory had commented on an exhibit of specimens of *Clathrus ruber* Fr. which he had recently collected in the Scilly Isles, Mr A. A. Pearson gave an interesting account of a visit to South Africa to study the agarics of Cape Province.

Tuesday's excursion to Clandeboye Demesne provided some of the best collecting of the week. At the start, fine clumps of *Coprinus comatus* and the less common *C. sterquilinus* attracted the photographers, and later *Microglossum viride* growing in abundance under beech was unfamiliar to most members and an apparently undescribed species of *Puccinia* was collected on *Pedicularis*. Before leaving Clandeboye, Helen's Tower (famous for its associations with Tennyson) was ascended and on the return journey there was a halt for tea at The Old Inn, Crawfordsburn.

A free morning on the Wednesday allowed time for a more critical examination of the collections laid out in the laboratories and gave members

AUTUMN FORAY (NORTHERN IRELAND) 11–17 SEPTEMBER 1948

an opportunity to study the exhibits illustrating the work of the Department. In the afternoon a group photograph was taken and later members were received by the Chancellor of the University and entertained to tea in the Great Hall. A 8 p.m. on the same day Prof. Muskett delivered his presidential address entitled 'Seed-borne fungi and their significance'.

On Thursday there was another whole-day excursion, first to Saintfield House, where among other finds a troop of *Panaeolus subbalteatus* on a pile of grass cuttings attracted attention, and then to Rademon Demesne where the grounds yielded additional records including one, *Tricholoma persicinum*, new for the British Isles. In the evening the Society was entertained by the Government of Northern Ireland to a dinner which was presided over by the Minister of Labour in the unavoidable absence of the Minister of Agriculture.

The final excursion was a joint one with the Advisory Plant Pathologists (who had been holding their Advisory Conference in Belfast on the preceding two days) to the Agricultural Research Institution for Northern Ireland at Hillsborough, Co. Down, where one party paid special attention to diseases of flax while another collected in the neighbouring woods.

The detailed local arrangements for this well-organized foray were made by Dr J. H. Colhoun and everyone present, but particularly the Foray Secretary, greatly appreciated all that Dr Colhoun and Prof. Muskett and their colleagues in the Department did to make the foray such a success.

The Society was indebted for permission to visit their property to the Marchioness of Dufferin and Ava, Col. Percival Price, Lady Reid, the Belvoir Golf Club, and the Trustees of the Agricultural Research Institution for Northern Ireland.

Thanks are also due to all those who supplied records and checked identifications in connexion with the list of species, and particularly to Mr E. N. Carrothers, Dr W. R. Ivimey Cook, Dr P. O'Connor, Dr R. W. G. Dennis, Madame Le Gal, Prof. A. E. Muskett, and Mr A. A. Pearson for their important contributions.

List of Species

B. = Belvoir Park, Co. Down; *C.* = Clandeboye Demesne, Co. Down; *G.* = Glenariff, Co. Antrim; *H.* = Hillsborough, Co. Down; *R.* = Rademon Demesne, Co. Down; S. = Saintfield House, Co. Down; *T.* = Tollymore Park, Co. Down.

* New record for Ireland. † New record for British Isles.

Names without author citations for Agaricaceae and Boletaceae are taken from Pearson & Dennis, *Trans. Brit. mycol. Soc.* **31**, 145–190, 1948, those for other Hymnomycetes and for Gasteromycetes from Rea, *British Basidiomycetae*, 1922.

BASIDIOMYCETES

Hymenomycetes

Amanita excelsa, *R.*, *S.*, *C.*, muscaria, *T.*, *C.*, pantherina, *T.*, *C.*, phalloides, *B.*, rubescens, *S.*, *H.*, *C.*, *B.*, *T.*, *R.*, virosa, *B.*
Amanitopsis fulva, *B.*, *S.*, inaurata, *H.*, vaginata, *T.*, *R.*
Armillaria mellea, *B.*, *G.*, *T.*, *C.*, *S.*, *R.*, mucida, *B.*, *C.*, *S.*
Bolbitius vitellinus, *G.*
Cantharellus cibarius, *B.*, *T.*, *C.*, *S.*, tubaeformis, *C.*
Clitocybe flaccida, *R.*, fragrans, *G.*, *C.*, nebularis, *R.*
Clitopilus prunulus, *B.*, *T.*, *H.*, *R.*, *C.*, *S.*

Collybia butyracea, *S.*, *T.*, *G.*, *C.*, *H.*, cirrata, *T.*, distorta, *R.*, *H.*, maculata, *T.*, platyphylla, *B.*, radicata, *C.*, *S.*

Coprinus atramentarius, *G.*, *C.*, comatus, *C.*, micaceus, *B.*, *G.*, *C.*, *S.*, plicatilis, *C.*, *S.*, sterquilinus, *C.*

Cortinarius acutus, *S.*, albo-violaceus, *T.*, *R.*, anomalus, *B.*, *C.*, *R.*, anthracinus*, *C.*, armillatus, *C.*, *T.*, bolaris, *S.*, caerulescens, *R.*, caninus, *C.*, *S.*, cinnamomeus, *R.*, *S.*, *C.*, croceo-caeruleus, *T.*, decipiens, *S.*, duracinus, *S.*, elatior, *R.*, *S.*, *H.*, *C.*, *B.*, *T.*, flexipes, *G.*, hemitrichus, *G.*, *C.*, hinnuleus, *C.*, *R.*, largus, *C.*, leucopus, *B.*, *C.*, *S.*, *H.*, multiformis, *R.*, paleaceus, *C.*, *S.*, *R.*, pholideus, *T.*, purpurascens, *S.*, raphanoides*, *T.*, rigens, *R.*, saniosus*, *R.*, torvus, *C.*, *T.*, *S.*, *H.*, triumphans, *C.*, varius, *C.*, *T.*, uliginosus, *C.*

Crepidotus calolepis, *B.*, *C.*, *T.*, mollis, *B.*, *S.*, *C.*, variabilis, *S.*, *H.*

Crinipellis stipitarius*, *T.*

Entoloma jubatum, *C.*, nidorosum, *B.*, *C.*, *T.*, *S.*, porphyrophaeum, *B.*, *C.*, prunuloides, *C.*, rhodopolium, *R.*

Flammula carbonaria, *C.*, *T.*, sapinea, *B.*, *C.*, *T.*, *R.*

Galera hypnorum, *B.*, *C.*, *T.*, *S.*

Hebeloma crustuliniforme, *R.*, *T.*, *C.*, mesophaeum, *C.*, testaceum, *G.*, *C.*

Hygrophorus agathosmus*, *R.*, calyptraeformis, *S.*, ceraceus, *S.*, *C.*, chlorophanus, *B.*, *T.*, *R.*, coccineus, *C.*, *T.*, conicus, *C.*, *T.*, intermedius, *R.*, laetus, *S.*, nigrescens, *C.*, obrusseus, *R.*, penarius, *R.*, pratensis, *C.*, *T.*, psittacinus, *C.*, *R.*, *S.*, puniceus, *R.*, reai, *T.*, unguinosus, *B.*, *S.*, virgineus, *G.*, *C.*, *R.*, *S.*

Hypholoma candolleanum, *B.*, *C.*, *S.*, fasciculare, *B.*, *C.*, *T.*, *R.*, *S.*, hydrophilum, *T.*, *S.*, lachrymabundum, *C.*, sublateritium, *S.*, *H.*, velutinum, *B.*, *C.*, *T.*, *S.*

Inocybe calamistrata, *T.*, *R.*, cookei, *C.*, *T.*, eutheles, *B.*, *C.*, fastigiata, *T.*, geophylla, *C.*, *T.*, geophylla var. lilacina, *B.*, *G.*, *C.*, *T.*, *R.*, *H.*, godeyi, *T.*, napipes*, *B.*, *T.*, obscura, *T.*, petiginosa, *B.*, *C.*, pyriodora, *T.*, umbrina*, *B.*

Laccaria amethystina, *B.*, *C.*, *T.*, *S.*, *H.*, laccata, *B.*, *G.*, *C.*, *S.*, *R.*, *H.*, proxima*, *S.*

Lactarius blennius, *B.*, *C.*, *T.*, *R.*, camphoratus, *S.*, chrysorheus, *R.*, cimicarius, *C.*, cyathula, *B.*, deliciosus, *C.*, *R.*, fuliginosus, *T.*, *R.*, glycyosmus, *C.*, *S.*, mitissimus, *B.*, *C.*, *T.*, *R.*, *S.*, pallidus, *C.*, *T.*, *R.*, piperatus, *T.*, plumbeus, *C.*, pyrogalus, *T.*, *R.*, quietus, *B.*, *R.*, *S.*, *H.*, *C.*, serifluus, *T.*, subdulcis, *B.*, *R.*, *S.*, torminosus, *C.*, vellereus, *T.*, *C.*

Lentinus cochleatus, *T.*, *C.*

Lepiota amianthina, *B.*, *C.*, *S.*, cristata, *S.*, rhacodes, *B.*, *R.*, *H.*

Leptonia chalybaea, *T.*, lampropus, *C.*, sericella, *G.*, *C.*

Marasmius confluens, *B.*, *C.*, *R.*, *T.*, dryophilus, *T.*, oreadoides, *H.*, peronatus, *B.*, *C.*, *T.*, *R.*, *S.*, ramealis, *T.*, rotula, *T.*, *R.*

Mycena acicula, *G.*, *C.*, *H.*, ammoniaca, *C.*, chlorantha, *C.*, filopes, *B.*, flavo-alba, *T.*, galericulata, *T.*, *R.*, *S.*, galopus, *B.*, galopus var. nigra, *S.*, *H.*, haematopus, *S.*, metata, *T.*, polygramma, *T.*, pura, *B.*, *T.*, *R.*, *S.*, sanguinolenta, *B.*, speirea, *R.*, tenerrima, *B.*, vitilis, *T.*

Naucoria bohemica*, *B.*, cucumis, *B.*, *R.*

Nolanea icterina,* *R.*, infula, *G.*, staurospora, *C.*, *S.*

Nyctalis parasitica, *T.*

Omphalia fibula, *C.*, *S.*, fibula var. swartzii*, *R.*

Panaeolus campanulatus, *B.*, *T.*, campanulatus var. sphinctrinus, *B.*, *T.*, subbalteatus, *S.*

Panus torulosus, *T.*

Paxillus involutus, *B.*, *C.*, *H.*

Pholiota erebia, *B.*, flammans, *T.*, mutabilis, *B.*, *S.*, spectabilis, *B.*, *C.*, *G.*, squarrosa, *S.*, togularis, *S.*

Pleurotus ostreatus, *B.*, *R.*

Pluteus cervinus, *B.*, *T.*, *S.*, *H.*

Psalliota arvensis, *R.*, silvatica, *C.*, *T.*, silvicola, *R.*

Psathyrella atomata, *C.*, disseminata, *C.*, *R.*, gracilis, *B.*, *C.*, *T.*, pennata*, *G.*

Psilocybe elongata, *G.*, *C.*, semilanceata, *C.*, spadicea, *T.*

Russula adusta, *B.*, *T.*, aeruginea*, *B.*, atropurpurea, *B.*, *S.*, *T.*, brunneo-violacea, *B.*, caerulea, *C.*, cyanoxantha, *B.*, *G.*, *C.*, *T.*, *S.*, *H.*, delica, *R.*, *B.*, *C.*, *T.*, *S.*, drimeia, *C.*, *R.*, emetica, *B.*, *R.*, *S.*, *H.*, fallax, *C.*, farinipes*, *T.*, fellea, *B.*, *C.*, *T.*, *S.*, *R.*, *H.*, foetans, *T.*, *S.*, fragilis, *C.*, *S.*, *H.*, grisea, *S.*, *R.*, heterophylla, *T.*, laurocerasi, *B.*, *T.*, maculata*, *S.*, mairei*, *B.*, *T.*, *C.*, *S.*, melliolens*, *S.*, *R.*, nauseosa*, *G.*, nigricans, *B.*, *C.*, *T.*, *S.*, *R.*, ochroleuca, *B.*, *T.*, *C.*, *S.*, *R.*, *H.*, puellaris, *C.*, romellii*, *T.*, rosea, *T.*, sanguinea, *C.*, sororia, *S.*, *R.*, venosa*, *C.*, vesca, *B.*, xerampelina, *T.*, xerampilina var. fusca*, *T.*

Stropharia aeruginosa, *B.*, *T.*, *C.*, *S.*, *R.*, semiglobata, *C.*, *S.*
Tricholoma acerbum, *H.*, album, *T.*, *C.*, *R.*, atrocinereum*, *C.*, carneum, *T.*, columbetta, *T.*, *S.*, fulvum, *G.*, *T.*, *C.*, immundum, *H.*, leucocephalum, *T.*, melaleucum, *T.*, *C.*, persicinum† Fr., *R.*, rutilans, *G.*, *T.*, *S.*, *R.*, *H.*, saponaceum var. squamosum, *T.*, *H.*, scalpturatum, *C.*, sulphureum, *R.*, terreum, *T.*, *C.*, *S.*, *R.*, ustale, *B.*, *C.*, *S.*, *R.*, virgatum, *C.*
Tubaria furfuracea, *B.*
Boletus albidus*, *B.*, badius, *C.*, *S.*, calopus, *T.*, *C.*, *S.*, carpini*, *B.*, *C.*, chrysenteron, *B.*, *T.*, *S.*, edulis, *B.*, *G.*, *T.*, *C.*, *S.*, elegans, *T.*, *C.*, erythropus, *B.*, *T.*, *C.*, *S.*, *R.*, luridus, *B.*, piperatus, *T.*, scaber, *B.*, *C.*, *S.*, subtomentosus, *B.*, *T.*, *C.*, *G.*
Daedalea biennis, *C.*, quercina, *B.*, *T.*
Fistulina hepatica, *B.*, *G.*, *T.*, *C.*, *S.*
Fomes annosus, *B.*, *T.*
Ganoderma applanatum, *B.*, *C.*, *S.*, *H.*
Irpex obliquus, *T.*
Merulius lacrymans, *T.*, rufus, *S.*
Polyporus adustus, *B.*, *T.*, *H.*, betulinus, *C.*, caesius, *T.*, giganteus, *B.*, *T.*, *S.*, *H.*, squamosus, *B.*, *S.*, varius, *C.*
Polystictus abietinus, *C.*, versicolor, *B.*, *T.*, *C.*
Poria sanguinolenta, *B.*
Trametes gibbosa, *B.*, *T.*, mollis, *B.*, *C.*
Acia uda, *T.*
Hydnum repandum, *B.*, *T.*, *S.*, *G.*, *C.*, repandum var. rufescens, *T.*
Clavaria abietina, *T.*, acuta, *G.*, amethystina, *C.*, cinerea, *G.*, *T.*, *C.*, corniculata, *T.*, *R.*, cristata, *C.*, fumosa, *S.*, *R.*, fusiformis, *T.*, inaequalis, *R.*, rugosa, *B.*, *G.*, *C.*, *R.*, stricta, *T.*, vermicularis, *C.*, *H.*
Corticium fusiforme*, *C.*, sambuci, *T.*
Craterellus cornucopioides, *T.*
Peniophora cinerea, *T.*
Phylacteria terrestris, *B.*, *C.*
Solenia anomala, *S.*
Stereum hirsutum, *B.*, *C.*, purpureum, *S.*, rugosum, *T.*
Tomentella subfusca (Karst.) v. Höhn. & Litsch., *C.*
Calocera cornea, *B.*, *T.*, viscosa, *B.*, *C.*
Dacrymyces deliquescens, *C.*, *B.*, *G.*
Exidia nucleata, *C.*, thuretiana, *S.*
Auricularia auricula-judae, *B.*, *T.*
Tremella foliacea*, *C.*, frondosa, *C.*, mesenterica, *B.*, *T.*, *C.*, *H.*
Tremellodon gelatinosum, *C.*

Gasteromycetes

Cyathus olla, *T.*
Cynophallus caninus, *B.*, *T.*
Lycoperdon perlatum, *B.*, *G.*, *S.*, *H.*, *T.*, pyriforme, *B.*, *H.*, *R.*, *T.*, *C.*, saccatum, *B.*, *S.*, umbrinum, *B.*
Phallus impudicus, *B.*
Scleroderma aurantium, *B.*, *C.*, *S.*, verrucosum, *B.*

Uredinales

Coleosporium euphrasiae Wint., *T.*, *H.*, melampyri Karst., *T.*, senecionis Fr., *T.*, sonchi Lév., *T.*, *C.*, *R.*, tussilaginis Tul., *B.*, *G.*, *T.*, *C.*, *H.*
Kuehneola albida Magn., *B.*, *C.*
Melampsora hypericorum Wint., *R.*, *T.*, lini Desm., *H.*
Melampsoridium betulinum Kleb., *B.*, *T.*, *C.*, *S.*
Milesina blechni Syd., *T.*, *C.*, murariae (Magn.) Faull, *C.*, *H.*, scolopendrii (Fuck.) Arth. *T.*, *C.*, *R.*
Phragmidium fragariastri Schröt., *G.*, rubi Wint., *T.*, rubi-idaei Karst., *G.*, *C.*, *R.*, violaceum, Wint., *B.*, *C.*, *S.*
Puccinia annularis Schl., *T.*, centaureae DC., *G.*, *T.*, circaeae Pers., *B.*, *C.*, expansa Link, *C.*, graminis Pers., on *Agrostis*, *T.*, hieracii Mart., *B.*, iridis Wallr., *B.*, lapsanae Fuckel, *G.*, *B.*, menthae Pers. *C.*, *S.*, *R.*, obscura Schröt., *B.*, obtegens Tul., *T.*, *C.*,

Puccinia poarum Niels., on *Tussilago*, *B.*, *G.*, *T.*, *R.*, primulae Duby, *G.*, pruni-spinosae Pers., *T.*, pulverulenta Grev., *S.*, saniculae Grev. *G.*, *T.*, *C.*, *R.*, sonchi Rob., *T.*, *R.*, taraxaci Plowr., *T.*, veronicae Schröt., *T.*, violae DC., *C.*
Pucciniastrum circaeae Speg., *B.*, *S.*, *R.*
Uromyces fabae de Bary, *G.*, rumicis Wint., *C.*

Ustilaginales

Sphacelotheca hydropiperis (Schum.) de Bary, *T.*, *C.*, *R.*
Tilletia holci (Westend.) Schröt., *B.*
Urocystis anemones (Pers.) Wint., on *Ranunculus*, *B.*, *C.*, *S.*, *H.*, violae (Sow.) Fisch. v. Wald., *C.*
Ustilago anomala* Kunze, on *Polygonum persicariae*, *H.*, succissae Magn., *C.*

Tuberales

Tuber puberulum* B. & Br., *G.*, *T.*, *S.*

Pyrenomycetes

Claviceps purpurea (Fr.) Tul., on *Anthoxanthum*, *Arrhenatherum*, *T.*, *Dactylis*, *Holcus*, *B.*, *T.*, *S.*, *Lolium*, *B.*, *Phleum*, *T.*
Cordyceps militaris (Fr.) Link, *T.*, *S.*, ophioglossoides (Fr.) Link, on *Elaphomyces*, *G.*
Cryptosphaeria eunomia* (Fr.) Fuckel, on *Fraxinus*, *B.*
Dialonectria peziza (Fr.) Cooke, *B.*
Diatrype stigma (Fr.) Fr., *B.*
Diatrypella favacea (Fr.) Ces. & de Not., *B.*
Epichloe typhina (Fr.) Tul., on *Bromus*, *T.*
Erysiphe cichoracearum DC., on *Hieracium pilosella*, *B.*, polygoni DC., on *Circea lutetiana*, *B.*, *T.*, *C.*, *Trifolium dubium*, *T.*, umbelliferarum de Bary, on *Heracleum sphondylium*, *B.*, *S.*, *C.*
Hypoxylon fuscum (Fr.) Fr., *B.*, rubiginosum (Fr.) Fr., on *Fagus*, *T.*
Melannoma pulvis-pyrius (Fr.) Fuckel, *B.*
Microsphaeria grossulariae (Wallr.) Lév., *T.*
Oidium quercinum Thüm., *C.*
Uncinula aceris (DC.) Sacc., *B.*, *H.*
Naumovia abundans Dobrozr., on *Prunella*, *G.*, *S.*
Nectria cinnabarina (Fr.) Fr., *B.*, *T.*, *H.*
Onygena corvina* Fr., *S.*
Ophiobolus graminis (Sacc.) Sacc. var. avenae Turner, *R.*
Phyllachora graminis (Fr.) Fuckel, *G.*, *T.*, *S.*
Pleospora herbarum (Fr.) Rabenh., on broom, *T.*
Stigmatea robertiani (Fr.) Fr., *G.*, *B.*
Ustulina vulgaris Tul., *B.*, *T.*
Xylaria carpophila (Fr.) Fr., *B.*, *T.*, hypoxylon (Fr.) Grev., *B.*, *C.*, *R.*, *H.*, longipes* Nits., *C.*, *R.*, *S.*
Venturia inaequalis (Cooke) Wint., *T.*

Discomycetes

Anthrocobia melaloma* Alb. & Schw., *S.*
Ascobolus furfuraceus Pers., *C.*
Ascophanus carneus (Pers.) Boud., on hay, *C.*, *H.*
Bulgaria inquinans (Pers.) Fr., *G.*, *R.*, *H.*
Catinella olivacea* (Batsch.) Boud., *C.*
Cheilymenea stercorea (Fr.) Boud., *T.*
Chlorosplenium aeruginosum (Oed. ex Fr.) De Not., *C.*, *T.*, *S.*, *R.*
Ciliaria umbrorum (Fr.) Boud., *T.*, scutellata (L. ex Fr.) Quél., *T.*, *B.*, hirta (Schum.) Boud., *C.*
Coryne sarcoides (Jacq.) Tul., *B.*, *C.*, *S.*, *H.*
Dasyscypha brevipila* Le Gal, *C.*, *T.*, cerinea (Pers. ex Fr.) Fuckel, *C.*, ciliaris (Schrad.) Sacc., on leaves of *Quercus* and *Castanea*, *C.*, diminuta* (Rob.) Sacc., on *Juncus*, *B.*, fuscescens* (Pers.) Rehm., *C.*, virginea (Batsch ex Fr.) Fuckel, on *Rubus fruticosus*, *C.*, on *Pseudotsuga*, *H.*

Fabraea ranunculi Karst., *C.*, *T.*
Galactinia badia (Pers.) Boud., *B.S.*, succosa (Berk.) Sacc., *B.*, *H.*, *G.*
Helotium aureum (Fr.) Fr., *C.*, phyllophyllum (Desm.) Karst., on *Fagus* leaf, *T.*, salicellum* (Fr.) Fr., on *Salix* twigs, *H.*, separabile* Karst., on *Rubus idaeus*, *H.* sulphuratum* (Fr.) Phill., *S.* (possibly H. epiphyllum, on pine needles instead of deciduous leaves), ciliatosporum (Fuck.), Boud., *H.*, herbarum, Fr. sensu karst., *T.*
Helvella crispa (Scop.) Fr., *T.*, sulcata Afz., *T.*, *C.*, *S.*
Keithia thujina (Dur.) Sacc., *T.*
Lachnea hemispherica (Wigg.) Gill., *B.*
Leotia lubrica (Scop.) Pers., *G.*, *T.*, *C.*, *R.*
Macropodia macropus (Pers. ex Fr.) Fuckel, *B.*, *G.*
Microglossum viride (Pers. ex Fr.) Gill., *C.*, *S.*
Mollisia amenticola* (Sacc.) Rehm, on *Alnus* catkins, *B.*, *C.*, discolor (Fr.) Phil. var. longispora* Le Gal, on *Quercus*, *C.*, ramealis* (Karst.) Karst., *C.*
Orbilia sarrayiniana Boud., *H.*, xanthostigma Fr., *C.*
Otidea alutacea (Pers. ex Fr.) Mass., *C.*, grandis* (Pers. ex Fr.) Mass., Co. Down, leporina (Batsch ex Fr.) Fuckel, *S.*, onotica Pers., *C.*
Peziza aurantia Pers., *B.*, *T.*, *C.*, *S.*
Phacidium multivalve (DC.) Kunze & Schm., *B.*, *T.*, *S.*
Phialea cyathoidea* (Bull. ex Fr.) Rehm., *G.*
Pseudopeziza ranunculi (Wallr.) Fuckel, *T.*
Rhytisma acerinum Fr., *B.*, *T.*, *C.*, *R.*
Sclerotinia curreyana (Berk.) Karst., *B.*
Rutstroemeria sydowiana (Rehm) White, *C.*, firma (Pers.) karst., *T.*
Spathularia flavida Pers. ex Fr., *B.*, *G.*, *T.*, *H.*
Stegia ilicis Fr., *B.*, *T.*, *S.*
Taphrina tosquineti (West.) Magn., *B.*, *S.*
Trichophaea woolhopeia* (Cooke & Phil.) Boud., *B.*
Trochila laurocerasi (Desm.) Fr., *R.*, laurocerasi var. smaragdina* (Lév.) Sacc., on *Prunus lusitanica*, *T.*
Tympanis conspersa* Fr., on *Sorbus aucuparia*, *B.*
Unguicularia scrupulosa* (Karst.) von Höhn., on *Rubus idaeus*, *H.*
Venturia inaequalis (Cooke) Winter, *T.*

Taphrinales

Protomyces microsporus Ung., *B.*, on *Aegopodium*, *T.*, *R.*

PHYCOMYCETES

Bremia lactucae Regel, on *Sonchus*, *R.*
Cystopus candidus (Pers.) de Bary, on *Cochlearia officinale*, Portrush.
Peronospora alsinearum Casp., *C.*, alta Fuckel, *T.*, grisea Unger, *B.*, verna Gäum. on *Veronica serpyllifolia*, *G*, Portrush.
Phycomyces nitens (Agardh) Kunze, *R.*
Phytophthora infestans (Mont.) de Bary, *T.*, *R.*, *H.*
Plasmopara densa Schröt., on *Bartsia*, *H.*
Synchytrium taraxaci de Bary & Woron., Portrush.

FUNGI IMPERFECTI

Alatospora acuminata Ing., *B.*, *G.*, *T.*, *C.*, *S.*
Anguillospora flagellifera† Ing., *C.*, longissima* Ing., *B.*, *C.*, *S.*
Articulospora tetracladia* Ing., *G.*, *T.*, *C.*
Ascochyta viciae* Lib., *T.*
Bispora monilioides Corda, *R.*
Clavariopsis aquatica de Wild., *B.*, *G.*, *S.*
Discella strobilina Died., *R.*
Didymaria didyma (Ung.) Schröt., *S.*
Flagellospora curvula Ing., *B.*, *G.*, *T.*, *C.*, *S.*
Heliscus aquaticus Ing., Crawfordsburn, Co. Down, longibrachiatus* Ing., *C.*
Lunulospora acuminata Ing., *B.*

Lemonniera aquatica de Wild., *B.*, *G.*, *T.*, *C.*, *S.*
Margaritispora aquatica* Ing., *C.*, *S.*
Marssonina potentillae Sacc., *C.*
Mastigosporium album Riess, *B.*
Ovularia obliqua (Cooke) Oud., *T.*, *C.*, *S.*, *R.*, sphaeroidea Sacc., *T.*, *S.*
Phleospora aegopodii Desm., *T.*, *R.*
Phoma urticae Sch. & Sacc., *C.*
Phyllosticta hedericola Dur. & Mont., *G.*, *S.*, *R.*
Polythrincium trifolii Kunze & Schm., *T.*
Ramularia agrestis* Sacc., *T.*, lactea (Desm.) Sacc., on *Viola canina*, *G.*, lapsanae (Desm.) Sacc., plantaginis Ellis & Martin, *B.*, primulae Thüm., *C.*, scrophulariae Fautr. & Roum., *T.*, *S.*, succisae Sacc., *G.*, taraxaci Karst., Portrush, Co. Antrim, ulmariae Cooke, *C.*
Sepedonium chrysospermum Fr., *C.*
Septoria polygonorum Desm., *S.*, *R.*, *H.*, ulmi* Died., *S.*
Spicaria farinosa (Holms.) Vuill., *B.*
Tetrachaetum elegans Ing., *B.*, *T.*, *S.*, marchalianum de Wild., *B.*, *G.*, *C.*, *S.*, setigerum Ing., *C.*
Tricladium gracile* Ing., *T.*, *C.*, splendens* Ing., *B.*, *G.*, *T.*, *C.*, *S.*

MYXOMYCETES

(Recorded by W. R. Ivimey Cook)

Arcyria denudata Sheldon, *C.*, *R.*, incarnata Pers., *T.*
Ceratomyxa fruticulosa Schröt., *B.*, *G.*, *T.*, *C.*
Colloderma oculatum G. Lister, *B.*
Cribraria argillacea Pers., *C.*, vulgaris Schrad., *C.*
Diderma effusum Morg., *R.*, spumarioides Fr., *C.*, *H.*
Didymium clavus Rost., *C.*, melanospermum Macbr., *T.*, nigripes Fr., *T.*, *C.*, *H.*
Fuligo septica Gmel., *B.*, *G.*, *T.*
Lycogala epidendrum Adans., *C.*, *R.*
Oliginema nitens Rost., *C.*
Physarum luteo-album Lister, *B.*, pusillum Lister, *T.*, virescens Ditm., *C.*
Stemonitis fusca Roth., *H.* splendens var. flaccida Lister, *C.*, *H.*
Trichia botrytis Pers., *T.*, *C.*, contorta Rost., *H.*, favoginea Pers., *R.*, varia Pers., *H.*, verrucosa Berk., *H.*
Tubifera ferruginosa Gmel., *C.*

AUTUMN DAY FORAYS, 1948

26 September.	Wendover, Bucks.
3 October.	Bookham Common, Surrey.
9 October.	Epping Forest, Essex.
	Bangor, Caern.
10 October.	Wyre Forest, Worcs.
16 October.	Ruislip Woods, Middlesex.
23 October.	Juniper Hall Field Centre, Box Hill, Surrey.

THE TWENTY-SECOND ANNUAL PLANT PATHOLOGY FIELD DAY

16 July 1948

The Twenty-second Annual Field Day was held at Cambridge. About seventy members of the Society met at the Botany School Field Station at 11 a.m.

Prof. F. T. Brooks gave an account of the history of the station, following which members split up into three parties and made a tour of the station. Particular interest was shown in the wheat plots used by Mr J. G. Manners in his investigation of races of *Puccinia glumarum* and Mr E. G. Bollard's demonstration of *Mastigosporium* spp. on grasses. The party then visited the Plant Virus Research Station by invitation of Dr Kenneth Smith. Here a brief tour of the Station was made and the remainder of the time was spent in examining the interesting exhibits set out. In the short time available members also took the opportunity of discussing their particular problems with Dr Smith and his staff.

A sandwich lunch was kindly provided by Prof. Brooks at the Botany School Field Station.

In the afternoon members split up into two parties to visit the Plant Breeding Institute, University Farm, by invitation of Dr G. D. H. Bell, and the Commonwealth Potato Station by invitation of Dr P. S. Hudson. At both institutions excellent demonstrations were provided by members of the staff. The only regret was that it was not possible to spend longer at each place.

The members present were again the guests of Prof. Brooks at tea at the Botany School Field Station.

After tea the Chairman of the Plant Pathology Committee, Mr R. W. Marsh, thanked Prof. Brooks for his hospitality and Dr W. J. Dowson for the excellent arrangements that had been made.

H. E. Croxall
Secretary, Plant Pathology Committee

REVIEWS

Les Agaricales; Agaricaceae. By P. Konrad and A. Maublanc. *Encyclopédie Mycologique*, vol. xiv. (Paris: Paul Lechevalier, 1948.) 469 pp.

It is refreshing to find a work on the Agarics which strikes a new note and promises to be of immense value. The book under review is not a flora in the usual sense of the word. It does not contain long descriptions and is not intended for the beginner. It is the first part, dealing with the family Agaricaceae only, of a work which aims at condensing the researches of recent years and presenting to the reader the species which the authors recognize as 'good', arranged in accordance with modern ideas. 'Phantom' species—species known only from a single record and insufficiently described—have been omitted, as are also unimportant synonyms and long detailed descriptions which can be found elsewhere. The intentions of the authors can be summarized by quoting two passages. First: 'Il ne faut pas oublier qu'il y a dans les livres beaucoup plus d'espèces...que dans la nature.' Secondly: 'Il est nécessaire de choisir un système logique et raisonable et d'éviter deux écueils: celui d'être réactionnaire et celui d'être révolutionnaire...Il ne faut pas accepter sans contrôle et sans que l'usage les ait admis toutes les nouveautés qui voient le jour.'

The work is divided into two parts. In the first or general part two chapters deal with the edibility of fungi and with the classification of poisons according to their effects, while a third chapter gives the history of the classification of the Agaricales briefly from the *Systema Mycologicum* of Fries (1821) to the present day, and ends with a summary of the grouping of the Basidiomycetes adopted by the authors. The second and main part of the work enumerates all the species of western and central Europe which the authors recognize. The classification adopted is a modification of that used in their *Icones Selectae Fungorum* (1924–1937), taking account of the researches of the past twenty years. The Agaricales are divided into six families, viz. Boletaceae, Paxillaceae, Gomphidiaceae, Hygrophoraceae, Russulaceae and Agaricaceae. As already stated the present volume deals only with the last family. The others are promised for a later volume.

Some genera which were taken up in the Icones are now dropped. Thus *Nematoloma* has gone and the name *Hypholoma* has been reinstated for species of the *H. sublateritium* section, a proceeding which seems to be not only common sense but is in accordance with the International Rules of Nomenclature.

The authors understand their species in a wide sense, ignoring many described varieties, subspecies, and even sometimes species which probably represent only ecologic forms or individual variations. Under each species they do not give a full description since this can be found elsewhere, but note the essential features, the habitat, and how the species may be distinguished from closely related forms. Spore measurements are not given unless they are essential as a point of distinction from another similar species. Only important synonyms are quoted, but finally, and most valuably, the authors give for each species references to figures in modern or at least easily accessible works which they consider to represent correctly the species in question. Thus under *Clitocybe phyllophila* we find that it is a species very close to *C. cerussata*, perhaps only a subspecies, differing in its more slender stature, more depressed cap and hollow stem; that *C. pithyophila* is the form found under conifers and that plate 139/81 of Cooke's *Illustrations* is good, as also Lange's figures and tab. 145 (*pithyophila*) of Bresadola's *Icones Mycologicae*, but *not* tab. 144 of Bresadola, which represents rather a small species of the *rivulosa- dealbata* group. Such notes are of the utmost value, based as they are on long experience in field work. The reviewer has had the book in constant use since its arrival, and it should certainly be in the hands of all serious students of the Agaricales. Naturally there will be differences of opinion as to some species. Not all students of *Mycena* will agree with some of Kühner's interpretations, which have been followed by Konrad and Maublanc. Nevertheless, it is possible to understand from this work what interpretation the authors do take, and how it differs from other possible views, and that is the essential point. It is to be hoped that the second volume will not be long delayed.

E. M. WAKEFIELD

Gasteromycetes of South Africa. By A. M. BOTTOMLEY. (*Bothalia*, vol. IV, part 3, 1948). Pp. 473–642, 79 black and white plates.

The Gasteromycetes have always been objects of interest. The large size of many species, their strange, even fantastic forms, and their occurrence often in dry, desert areas long ago excited the curiosity of early travellers, and odd specimens of quite rare species found their way into European herbaria. Successive mycologists have speculated as to their origin and relationships, and various systems of classification have been proposed. This latest work by Miss Bottomley on the South African Gasteromycetes does not, however, contribute anything new on these lines. She has painstakingly put together all the known records of these plants from South Africa, and has followed the classification used by Cunningham in his Gasteromycetes of Australia and New Zealand (see this *Journal*, vol. **28**, pp. 150–151, 1945). Keys are provided to families, genera and species, and each species is described in detail, and all South African records listed. The paper is illustrated by uncoloured plates reproducing photographs of the plants dealt with, but there are no drawings of microscopic details. The most outstanding feature is the large number of Gasteromycetes which have been recorded from this region; 143 species are here recognized, while there is mention of twenty more doubtful or unknown records. Out of this number forty-six species and three genera are known only from South Africa, and ten of the doubtful species are also recorded only from this region. Among the endemic species five are here described for the first time, namely *Lycoperdon duthiei*, *L. qudenii*, *Calvatia incerta*, *Bovista umbrina* and *Geastrum dissimile*. Rejection of Morgan's genus *Bovistella* necessitates two new combinations, *Bovista oblongispora* (Lloyd) Bottomley and *B. citrina* (B. &. Br.) Bottomley.

The work provides a good basis for the more critical survey of South African species which must eventually be undertaken. There are a number of typographical errors, mostly obvious. Some, but not all, have been indicated in a type-written slip enclosed with the number.

E. M. WAKEFIELD

Diseases of flax. By A. E. MUSKETT and JOHN COLHOUN. (Obtainable from the Bursar, The Queen's University, Belfast, 1947.) Price 21*s*.

The growing of fibre flax in the British Isles has long been centred in Ireland, particularly Northern Ireland, and there as elsewhere in the islands, the peak periods of production have been in times of national emergency. Between the two world wars the acreage devoted to the crop dropped very considerably, and the investigations of flax diseases, specially undertaken by the old Irish Department of Agriculture and Technical Instruction during, and for a short time after, the first World War, were allowed to lapse. Even before the outbreak of the 1939–45 War it was evident that the United Kingdom would have to become virtually self-sufficient in producing flax fibre and seed in the event of war. Problems of disease were inevitable, and at the request of the Northern Ireland Flax Development Committee, Prof. Muskett and his colleagues lost no time in seeking the answers to them. It was soon found that partial crop failures and unsatisfactory yields were often caused by seed-borne diseases. The diseases were carefully studied: laboratory methods were devised for testing flax seed for the presence of fungus parasites, thus laying the foundations for survey work and seed certification; and effective means of disinfecting contaminated seed before sowing were found and put into practice as a routine measure. Without doubt the relative freedom from disease of the flax crop during the war and since has been due in great measure to the foresight and industry shown by Northern Ireland: indeed, it has been stated that seed disinfection alone increased the value of the United Kingdom flax crop during the war years by some £2,000,000.

And now Prof. Muskett and Dr Colhoun have done a great service to plant pathologists and others, by summarizing with the covers of a book of 112 pages what is known about some thirty diseases to which flax is subject. There is a straightforward, readable general account of each disease followed by a section on its prevention and control, and by a list of most of the known references to it, the important ones being given in bold type. In addition there are four valuable appendices dealing respectively with the Ulster method used for examining flax seed for disease, seed disinfection, the production and certification of seed, and the growing of a healthy flax crop. There are ninety-one illustrations, of which

rather more than one-third are colour tinted, though the doubtful advantages gained by colouring scarcely justify the added expense of reproduction. The book is enriched and illumined throughout by the experience of ten years of research, and it is strongly recommended to all mycologists and plant pathologists. It should also be of value to all who cultivate flax or linseed.

W. C. MOORE

Fungi and Plant Disease. By B. B. MUNDKUR. (Macmillan and Co., 1949.) x + 246 pp. Price 16*s*.

Familiarity with fungi can come only through handling them, and little insight can be obtained into the nature of plant diseases without studying them in the field, but reading is an essential and primary phase of these processes. It is therefore a great advantage if the reader's attention can be directed to the diseases he is likely to find in his own surroundings, and that is what Dr Mundkur has done in a book intended primarily for undergraduate students of agriculture, horticulture and botany in Indian universities and agricultural colleges. The host plants so familiar to readers of text-books on plant diseases published in the temperate regions—books on which students in tropical and subtropical regions have for the most part also had to rely—take second place here to palms, rice, bajra, jowar, gram, sugar-cane and the like. In the same way the diseases described are mostly unfamiliar to British readers, and even with such well-known ones as Potato Blight and Loose Smut of wheat, the text reads strangely at times to the western eye, because emphasis is naturally and rightly placed on local conditions and practices. The author, who is on the staff of the Plant Diseases Division of the Ministry of Agriculture at New Delhi, writes authoritatively, and the book should also prove of interest and value in Britain for general reading and to students who are destined to work overseas.

The first three chapters provide an elementary introduction to fungi, their morphology, reproduction, metabolic processes, and the part they play in causing plant disease. These chapters comprise in effect a running descriptive glossary of the terms commonly used in mycology and plant pathology. Chapter 4, which describes methods of studying plant diseases, is inadequate and needs considerable expansion. The average student can soon master the technique of isolation but usually has no idea how to set about identifying the fungi he obtains. To be told merely that 'identification of the isolates then follows' (p. 36) is no more helpful than the bare statement (p. 40) that 'the same precision of aseptic technique must be employed that one would use in any careful mycological culture work'. The most valuable part of the book is contained in four chapters (150 pages) dealing respectively with the Phycomycetes, Ascomycetes, Basidiomycetes and Fungi Imperfecti. Keys to the orders and families of importance in plant pathology are included, and brief illustrated accounts are given of some forty fungus diseases of economic plants occurring in India. There are short chapters on bacterial and virus diseases of plants, which would be worth expanding (with an appropriate change in the title of the book), and a final chapter on plant disease control. No mention is made (p. 207) of Dowson's classification of bacteria or of his genus *Xanthomonas* which has now been generally accepted. For further reading, references are given at the end of each chapter and there is an adequate index. The format is attractive and the book is well illustrated.

W. C. MOORE

Observations, Botanical and Physiological, on the Potato Murrain. By M. J. BERKELEY. Together with Selections from Berkeley's *Vegetable Pathology*. Made by the Plant Pathology Committee of the British Mycological Society. Phytopathological Classics, No. 8, 104 pp., 6 plates, 1948. 1.50 dollars. (East Lansing, Mich., American Phytopathological Society.)

Scientific literature is ephemeral. Its classics are seldom read. Scientists build on the foundations laid by their predecessors and it is customary only to inspect these foundations when their condition is suspect. A measure of the importance of a scientific paper is often the rapidity with which the results described or the theories formulated are incorporated into scientific thought and the rapidity with which the paper itself comes to be consulted

only by the historian. It is, however, well that the student of any branch of science should be acquainted with the origin of concepts fundamental to his studies, and both mycologists and plant pathologists are further indebted to the American Phytopathological Society for this latest addition to Phytopathological Classics.

It may startle a modern reader that only one hundred years ago the relation of the fungus to the diseased plant in what is now accepted as such a clear example of parasitism as of *Phytophthora infestans* on the potato could be a live issue, and he may overlook the fact that Berkeley's conclusion 'the decay is the consequence of the presence of the mould, and not the mould of the decay' marked a turning point in the development of mycology. On the other hand, it is humbling to study the index to the notable series of articles on Vegetable Pathology which shows Berkeley to have had a clearer vision of plant pathology as a whole than had many of his successors. Only recently has the wheel turned full circle and plant pathology come to be recognized not as a mere study in symptomology or the careful description of plant pathogenic fungi but as the totality of the complex relationships of the pathogen and the growing plant to each other and to the environment.

Dr Ramsbottom has written an informative biographical note as a preface to this Classic in which the Linnean Society of London's portrait of Berkeley is reproduced as the frontispiece.

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